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(21) International Application Number: PCT/EP98/06977 (22) International Filing Date: 9 October 1998 (09.10.98) (30) Priority Data: PO 9745 10 October 1997 (10.10.97) AU (71) Applicant (for all designated States except US): RHONE-POULENC AGRO [FR/FR]; 14/20, rue Pierre Baizet, F-69009 Lyon (FR). (72) Inventors; and (75) Inventors/Applicants (for US only): DOUTRIAUX, Marie-Pascale [FR/FR]; 64, route de Villebon, F-91160 Saulx les Chartreux (FR). BETZNER, Andreas, Stefan [AU/AU]; 40 Dallachy Place, Page, ACT 2614 (AU). FREYSSINET, Georges [FR/FR]; 21, rue de Nervieux, F-69450 Saint Cyr au Mont d'Or (FR). PEREZ, Pascal [FR/FR]; 17, chemin de la Pradelle, Varennes, F-63450 Chanonat (FR). (74) Agent: GENIN, Patrick; Rhône-Poulenc Agro, DPI, 14/20, rue Pierre Baizet, F-69009 Lyon (FR).	(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG). Published Without international search report and to be republished upon receipt of that report.	
(54) Title: METHODS FOR OBTAINING PLANT VARIETIES		
(57) Abstract An isolated and purified DNA molecule comprising a polynucleotide sequence encoding a polypeptide functionally involved in the DNA mismatch repair system of a plant.		

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Methods for Obtaining Plant Varieties

TECHNICAL FIELD

The present invention relates to nucleotide sequences which encode polypeptides involved in the DNA mismatch repair systems of plants, and to the polypeptides encoded
5 by those nucleotide sequences. The invention also relates to nucleotide sequences and polypeptide sequences for use in altering the DNA mismatch repair system in plants. The invention also relates to a process for altering the DNA mismatch repair system of a plant cell, to a process for increasing genetic variations in plants and to processes for obtaining plants having a desired characteristic.

10

BACKGROUND OF THE INVENTION

Plant breeding essentially relies on and makes use of genetic variation which occurs naturally within and between members of a family, a genus, a species or a subspecies. Another source of genetic variation is the introduction of genes from other organisms which may or may not be related to the host plant.

15 Allelic loci or non-allelic genes which constitute or contribute to desired quantitative (e.g. growth performance, yield, etc.) or qualitative (e.g. deposition, content and composition of seed storage products; pathogen resistance genes; etc.) traits that are absent, incomplete or inefficient in a species or subspecies of interest are typically introduced by the plant breeder from other species or subspecies, or *de novo*. This
20 introduction is often done by crossing, provided that the species to be crossed are sexually compatible. Other means of introducing genomes, individual chromosomes or genes into plant cells or plants are well known in the art. They include cell fusion, chemically aided transfection (Schocher et al., 1986, Biotechnology 4: 1093) and ballistic (McCabe et al., 1988, Biotechnology 6: 923), microinjection (Neuhaus et al., 1987, TAG 75: 30),
25 electroporation of protoplasts (Chupeau et al., 1989, Biotechnology 7: 53) or microbial transformation methods such as *Agrobacterium* mediated transformation (Horsch et al., 1985, Science 227: 1229; Hiei et al., 1996, Biotechnology 14: 745).

However, when a foreign genome, chromosome or gene is introduced into a plant, it will often segregate in subsequent generations from the genome of the recipient plant or
30 plant cell during mitotic and meiotic cell divisions and, in consequence, become lost from the host plant or plant cell into which it had been introduced. Occasionally, however, the introduced genome, chromosome or gene physically combines entirely or in part with the genome, chromosome or gene of the host plant or plant cell in a process which is called recombination.

35 Recombination involves the exchange of covalent linkages between DNA molecules in regions of identical or similar sequence. It is referred to here as homologous recombination if donor and recipient DNA are identical or nearly identical (at least 99%

base sequence identity), and as homeologous recombination if donor and recipient DNA are not identical but are similar (less than 99% base sequence identity).

The ability of two genomes, chromosomes or genes to recombine is known to depend largely on the evolutionary relation between them and thus on the degree of sequence similarity between the two DNA molecules. Whereas homologous recombination is frequently observed during mitosis and meiosis, homeologous recombination is rarely or never seen.

From a breeder's perspective, the limits within which homologous recombination occurs, therefore, define a genetic barrier between species, varieties or lines, in contrast to homeologous recombination which can break this barrier. Homeologous recombination is thus of great importance for plant breeding. Accordingly there is a need for a process for enhancing the frequency of homeologous recombination in plants. In particular, there is a need for a process of increasing homeologous recombination to significantly shorten the length of breeding programs by reducing the number of crosses required to obtain an otherwise rare recombination event.

At least in *Escherichia coli*, homologous and homeologous recombination are known to share a common pathway that requires among others the proteins RecA, RecB, RecC, RecD and makes use of the SOS induced RuvA and RuvB, respectively. It has been suggested that mating induced recombination follows the Double-Strand Break Repair model (Szostak et al., 1983, Cell 33, 25-35), which is widely used to describe genetic recombination in eukaryotes. Following the alignment of homologous or homeologous DNA double helices the RecA protein mediates an exchange of a single DNA strand from the donor helix to the aligned recipient DNA helix. The incoming strand screens the recipient helix for sequence complementarity, seeking to form a heteroduplex by hydrogen bonding the complementary strand. The displaced homologous or homeologous strand of the recipient helix is guided into the donor helix where it base pairs with its counterpart strand to form a second heteroduplex. The resulting branch point then migrates along the aligned chromosomes thereby elongating and thus stabilising the initial heteroduplexes. Single stranded gaps (if present) are closed by DNA synthesis. The strand cross overs (Holliday junction) are eventually resolved enzymatically to yield the recombination products.

Although in wild type *E. coli* homologous and homeologous recombination are thus mechanistically similar if not identical, homologous recombination in conjugational crosses *E. coli* x *E. coli* occurs five orders of magnitude more frequently than homeologous recombination in conjugational crosses *E. coli* x *S. typhimurium* (Matic et al. 1995; Cell 80, 507-515). The imbalance in favour of homologous recombination was shown to be caused largely by the bacterial MisMatch Repair (MMR) system since its

inactivation increased the frequency of homeologous recombination in *E. coli* up to 1000 fold (Rayssiguier et al. 1989, Nature 342, 396-401).

In *E. coli*, the MMR system (reviewed by Modrich 1991, Annual Rev Genetics 25, 229-253) is composed of only three proteins known as MutS, MutL and MutH. MutS
5 recognizes and binds to base pair mismatches. MutL then forms a stable complex with mismatch bound MutS. This protein complex now activates the MutH intrinsic single stranded endonuclease which nicks the strand containing the misplaced base and thereby prepares the template for DNA repair enzymes.

During recombination, MMR components inhibit homeologous recombination. In
10 vitro experiments demonstrated that MutS in complex with MutL binds to mismatches at the recombination branch point and physically blocks RecA mediated strand exchange and heteroduplex formation (Worth et al., 1994; PNAS 91, 3238-3241). Interestingly, the SOS dependent RuvAB mediated branch migration is insensitive to MutS/MutL, explaining the observed slight increase in SOS dependent homeologous recombination.
15 Homeologous mating even induces the SOS response, thereby taking advantage of RuvAB induction (Matic et al. 1995, Cell 80, 507-515).

The MMR system thus appears to be a genetic guardian over genome stability in *E. coli*. In this role it essentially determines the extent to which genetic isolation, that is, speciation, occurs. The diminished sensitivity of the SOS system to MMR, however,
20 allows (within limits) for rapid genomic changes at times of stress, providing the means for fast adaptation to altered environmental conditions and thus contributing to intraspecies genetic variation and species evolution.

The important role of MMR in preserving genomic integrity has been established also in certain eukaryotes. In its efficiency, the human MMR, for example, may even
25 counteract potential gene therapy tools such as triple-helix forming oligonucleotides including RNA-DNA hybrid molecules (Havre et al., 1993, J. Virology 67: 7234-7331; Wang et al., 1995, Mol. Cell. Biol. 15: 1759-1768; Kotani et al., 1996, Mol. Gen. Genetics 250: 626-634; Cole-Strauss et al., 1996, Science 273: 1387-1389). Such oligonucleotides are designed to introduce single base changes into selected DNA target
30 sequences in order to inactivate for example cancer genes or to restore their normal function. The resulting base mismatches however are recognised by the mismatch repair system which then directs removal of the mismatched base, thereby reducing the efficiency of oligonucleotide induced site-specific mutagenesis.

To date, two families of related genes, homologous to the bacterial *MutS* and *MutL*
35 genes have been identified or isolated in yeast and mammals (recent reviews by Arnheim and Shibata, 1997, Curr. Opinion Genet. Dev. 7, 364-370; Modrich and Lahue, 1996, Annual Rev. Biochem. 65, 101-133; Umar and Kunkel, 1996, Eur. J. Biochem. 238, 297-307). Biochemical and genetic analysis indicated that eukaryotic MutS homologs (MSH)

and MutL homologs (MLH, PMS), respectively, fulfil similar protein functions as their bacterial counterparts. Their relative abundance, however, could reflect different mismatch specificity and/or specialisation for different tissues or organelles or developmental processes such as mitotic versus meiotic recombination.

5 To date, six different genes homologous to *MutS* have been isolated in yeast (*yMSH*), and their homologs have been found in mouse (*mMSH*) and human (*hMSH*), respectively. Encoded proteins *yMSH2*, *yMSH3* and *yMSH6* appear to be the main *MutS* homologs involved in MMR during mitosis and meiosis in yeast, where the complementary proteins *MSH3* and *MSH6* alternatively associate with *MSH2* to recognise
10 different mismatch substrates (Masischky et al., 1996, Genes Dev. 10, 407-420). Similar protein interactions have been demonstrated for the human homologs *hMSH2*, *hMSH3* and *hMSH6* (Acharya et al., 1996, PNAS 93, 13629-13634).

MutL homologs (MLH and PMS), recently reviewed by Modrich and Lahue (1996, Annual Rev. Biochem. 65, 101-133) have so far been found in yeast (*yMLH1* and
15 *yPMS1*), mouse (*mPMS2*) and human (*hMLH1*, *hPMS1* and *hPMS2*). The *hPMS2* is a member of a family of at least 7 genes (Horii et al., 1994, Biochem. Biophys. Res. Commun. 204, 1257-1264) and its gene product is most closely related to *yPMS1*. Prolla et al. (1994, Science 265, 1091-1093) presented evidence for *yPMS1* and *yMLH1* to physically associate with each other and, together, to interact with the *MutS* homolog
20 *yMSH2* to form a ternary complex involved in mismatch substrate binding.

However, while medical interest in mismatch repair has prompted extensive research on MMR in bacteria, yeast and mammals, MMR genes have not been isolated from higher plants prior to the present invention and no attempts to adjust the plant MMR to plant breeding needs have been reported.

25

SUMMARY OF THE INVENTION

According to a first embodiment of the invention, there is provided an isolated and purified DNA molecule comprising a polynucleotide sequence encoding a polypeptide functionally involved in the DNA mismatch repair system of a plant. In one form of this embodiment, the invention provides an isolated and purified DNA molecule comprising a
30 polynucleotide sequence encoding a polypeptide which is homologous to a mismatch repair polypeptide of a yeast or of a human. More particularly, the invention provides polynucleotide sequences encoding polypeptides which are homologous to the mismatch repair polypeptides *MSH3* and *MSH6* of *Saccharomyces cerevisiae*. Still more particularly, the invention provides the coding sequences of the genes *AtMSH3* and
35 *AtMSH6* of *Arabidopsis thaliana*, as defined hereinbelow, and polynucleotide sequences encoding polypeptides which are homologous to polypeptides encoded by *AtMSH3* and *AtMSH6*.

According to a second embodiment of the invention, there is provided an isolated and purified polypeptide functionally involved in the DNA mismatch repair system of a plant, for example a polypeptide which is homologous to a mismatch repair polypeptide of a yeast or of a human such as a polypeptide encoded by the genes *AtMSH3* or *AtMSH6* of
5 *Arabidopsis thaliana*, as defined hereinbelow.

According to a third embodiment of the invention, there is provided an isolated and purified DNA molecule comprising a polynucleotide sequence selected from the group consisting of (i) a sequence encoding a polynucleotide which is capable of interfering with the expression of a plant polynucleotide sequence encoding a polypeptide which is
10 homologous to a mismatch repair polypeptide of a yeast or of a human and thereby disabling said plant polynucleotide sequence; and (ii) a sequence encoding a polypeptide capable of disrupting the DNA mismatch repair system of a plant.

According to a fourth embodiment of the invention there is provided a chimeric gene comprising a DNA sequence selected from the group consisting of (i) a sequence encoding
15 a polynucleotide which is capable of interfering with the expression of a plant polynucleotide sequence encoding a polypeptide which is homologous to a mismatch repair polypeptide of a yeast or of a human and thereby disabling said plant polynucleotide sequence, and (ii) a sequence encoding a polypeptide capable of disrupting the DNA mismatch repair system of a plant: together with at least one regulation element capable of
20 functioning in a plant cell. Examples of such regulation elements include constitutive, inducible, tissue type specific and cell type specific promoters such as 35S, NOS, PR1a, AoPR1 and DMC1. Typically, a chimeric gene of the fourth embodiment will also include at least one terminator sequence, more typically exactly one terminator sequence.

In the third and fourth embodiments, said interference, by said polynucleotide
25 sequence, with the expression of a plant polynucleotide sequence encoding a polypeptide which is homologous to a mismatch repair peptide of a yeast or a human typically occurs by hybridisation or by co-suppression.

According to a fifth embodiment of the invention there is provided a plasmid or vector comprising a chimeric gene of the fourth embodiment. A vector of the fifth
30 embodiment may be, for example, a viral vector or a bacterial vector.

According to a sixth embodiment of the invention, there is provided a plant cell stably transformed, transfected or electroporated with a plasmid or vector of the fifth embodiment.

According to seventh embodiment of the invention, there is provided a plant
35 comprising a cell of the sixth embodiment.

According to an eighth embodiment of the invention, there is provided a process for at least partially inactivating a DNA mismatch repair system of a plant cell, comprising

transforming or transfecting said plant cell with a DNA sequence of the third embodiment or a chimeric gene of the fourth embodiment or a plasmid or vector of the fifth embodiment, and causing said DNA sequence to express said polynucleotide or said polypeptide.

5 According to a ninth embodiment of the invention, there is provided a process for increasing genetic variation in a plant comprising obtaining a hybrid plant from a first plant and a second plant, or cells thereof, said first and second plants being genetically different; altering the mismatch repair system in said hybrid plant; permitting said hybrid plant to self-fertilise and produce offspring plants; and screening said offspring plants for
10 plants in which homeologous recombination has occurred. For example, homeologous recombination may be evidenced by new genetic linkage of a desired characteristic trait or of a gene which contributes to a desired characteristic trait.

According to a tenth embodiment of the invention there is provided a process for obtaining a plant having a desired characteristic, comprising altering the mismatch repair
15 system in a plant, cell or plurality of cells of a plant which does not have said desired characteristic, permitting mutations to persist in said cells to produce mutated plant cells, deriving plants from said mutated plant cells, and screening said plants for a plant having said desired characteristic.

In a preferred form of the ninth and tenth embodiments of the invention, the step of
20 altering the mismatch repair system comprises introducing into said hybrid plant, plant, cell or cells a chimeric gene of the fourth embodiment and permitting the chimeric gene to express a polynucleotide which is capable of interfering with the expression of a plant polynucleotide sequence in a mismatch repair gene of the hybrid plant, plant, cell or cells, or a polypeptide capable of disrupting the DNA mismatch repair system of the hybrid
25 plant or cells.

In other embodiments, the invention provides (a) an oligonucleotide capable of hybridising at 45°C under standard PCR conditions to a DNA molecule of the first embodiment; (b) an oligonucleotide capable of hybridising at 45°C under standard PCR conditions to the DNA of SEQ ID NO: 18 and (c) an oligonucleotide capable of
30 hybridising at 45°C under standard PCR conditions to the DNA of SEQ ID NO:30; with the proviso that the oligonucleotide of (a), (b) and (c) is other than SEQ ID NO:1 or SEQ ID NO:2.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 provides a diagrammatic representation of the primer sequences used to
35 isolate *AtMSH3*.

Figure 2 is a plasmid map of clone 52, showing restriction enzyme cleavage sites in the 5' half of the full-length cDNA for *AtMSH3*.

Figure 3 is a plasmid map of clone 13, showing restriction enzyme cleavage sites in the 3' half of the full-length cDNA for *AtMSH3*.

Figure 4 is a sequence listing of the coding sequence of *AtMSH3*, together with a deduced sequence of the encoded polypeptide.

5 Figure 5 is a protein alignment of yeast (*Saccharomyces cerevisiae*) and *Arabidopsis thaliana* MSH3 protein.

Figure 6 provides a diagrammatic representation of the primer sequences used to isolate *AtMSH6*.

10 Figure 7 is a plasmid map of clone 43, showing restriction enzyme cleavage sites in the 5' half of the full-length cDNA for *AtMSH6*.

Figure 8 is a plasmid map of clone 62, showing restriction enzyme cleavage sites in the 3' half of the full-length cDNA for *AtMSH6*.

Figure 9 is a sequence listing of the coding sequence of *AtMSH6*, together with a deduced sequence of the encoded polypeptide.

15 Figure 10 is a protein alignment of yeast (*Saccharomyces cerevisiae*) and *Arabidopsis thaliana* MSH6 protein.

Figure 11 is a genomic sequence listing of *AtMSH6*.

Figure 12 is a plasmid map of plasmid pPF13.

Figure 13 is a plasmid map of plasmid pPF14.

20 Figure 14 is a plasmid map of plasmid pCW186.

Figure 15 is a plasmid map of plasmid pCW187.

Figure 16 is a plasmid map of plasmid pPF66.

Figure 17 is a plasmid map of plasmid pPF57.

Figure 18 is a diagrammatic representation of an antisense gene construction for use
25 in homeologous meiotic recombination.

Figure 19 is a plasmid map of plasmid p3243.

DETAILED DESCRIPTION OF THE INVENTION

The present invention is based on the inventors' discovery that there exist in higher plants genes which are homologous to MMR genes in *E. coli*, and to MMR genes in
30 yeasts and humans.

Thus, the inventors have identified genes, herein designated *AtMSH3* and *AtMSH6*, of the plant *Arabidopsis thaliana* which encode the proteins AtMSH3 and AtMSH6. These plant proteins are homologous to yMSH3 and yMSH6, respectively. The present inventors have isolated cDNAs encoding the proteins AtMSH3 and AtMSH6 and have
35 isolated the complete gene encoding AtMSH6. Given the teaching herein, other genes (for example AtMSH2, and genes of other plants) may be obtained which are involved in DNA mismatch repair in plants, including other genes which encode polypeptides homologous to MMR proteins of yeasts or humans, such as genes which encode

polypeptides homologous to yeast MSH2, MLH1 or PMS2, or to human MLH1, PMS1 or PMS2. For example, given the teaching herein, genes of members of the *Brassicaceae* family or of other unrelated families, for example the *Poaceae*, the *Solanaceae*, the *Asteraceae*, the *Malvaceae*, the *Fabaceae*, the *Linaceae*, the *Canabinaceae*, the *Dauaceae* 5 and the *Cucurbitaceae* family, and which encode polypeptides homologous to MMR proteins of yeasts or humans may be obtained.

Examples of plants whose genes encoding polypeptides homologous to MMR proteins of yeasts or humans may be obtained given the teaching herein include maize, wheat, oats, barley, rice, tomato, potato, tobacco, capsicum, sunflower, lettuce, 10 artichoke, safflower, cotton, okra, beans of many kinds including soybean, peas, melon, squash, cucumber, oilseed rape, broccoli, cauliflower, cabbage, flax, hemp, hops and carrot.

Within the meaning of the present invention, a first polypeptide is defined as homologous to a second polypeptide if the amino acid sequence of the first polypeptide 15 exhibits a similarity of at least 50% on the polypeptide level to the amino acid sequence of the second polypeptide.

A procedure which may be followed to obtain genes *AtMSH3* and *AtMSH6* is described in Example 1. Essentially the same technique may be applied to obtain other mismatch repair genes of *Arabidopsis thaliana*, and essentially the same technique as 20 exemplified herein may be applied to cDNA obtained by reverse transcription of RNA from other plants. Alternatively, given the sequence information disclosed herein, other degenerate oligonucleotide primers, especially oligonucleotides of the invention which are capable of hybridising at 45°C under standard PCR conditions (such as the conditions described in Example 1 using primers UPMU and DOMU) to *AtMSH3* and/or *AtMSH6* 25 may be designed and obtained for use in isolating sequences of plant mismatch repair genes which are homologous to *AtMSH3* or *AtMSH6*, from other plants. Similarly, oligonucleotides of the invention which are capable of hybridising at 45°C under standard PCR conditions to plant mismatch repair genes of plants other than *Arabidopsis thaliana* also fall within the scope of the present invention and may be utilised to obtain mismatch 30 repair genes of still other plants. Typically, such oligonucleotides are capable of hybridising at 45°C under standard PCR conditions to a DNA molecule which encodes a polypeptide which is homologous to a mismatch repair polypeptide of a yeast or a human. The temperature at which oligonucleotides of the invention hybridise to *AtMSH3* and/or *AtMSH6*, or to plant mismatch repair genes of plants other than *Arabidopsis thaliana*, or 35 to DNA molecules which encode polypeptides which are homologous to a mismatch repair polypeptide of a yeast or a human may be higher than 45°C, for example at least 50°C, or at least 55°C, or at least 60°C or as high as 65°C.

The successful gene isolation disclosed herein demonstrates for the first time the existence of MMR in higher plants and indicates the presence of other plant MMR genes. For example, genes encoding the plant homologs of MSH1, MSH2, MSH4, MSH5, PMS1, PMS2 and MLH1 may be identified given the teaching herein. Such genes, as well as those specifically described herein, separately or in combination, are useful in manipulating the plant MMR for plant breeding purposes. Thus, for example, the plant MMR may be altered by including in a plant cell a polynucleotide sequence as defined herein above with reference to the third embodiment of the invention, and causing the polynucleotide sequence to express either a polynucleotide which disables a plant MMR gene, or a polypeptide which disrupts the plant's MMR system.

The DNA molecule of the third embodiment of the invention includes a polynucleotide sequence (herein referred to as a MMR altering gene) which may for example encode sense, antisense or ribozyme molecules characterised by sufficient base sequence similarity or complementarity to the gene to be altered to permit the antisense or ribozyme molecule to hybridise with the plant MMR gene in vivo or to permit the sense molecule to participate in co-suppression. Alternatively, the MMR altering gene may encode a protein or proteins which interfere with the activity of a plant MMR protein and thus disrupt the plant's MMR system. For example, such encoded proteins may be antibodies or other proteins capable of interfering with MMR protein function, such as by complexing with a protein functionally involved in plant MMR thereby disrupting the MMR of the plant. An example of such a protein is the MSH3 protein of *Arabidopsis thaliana* described herein or a protein of another plant which is homologous to the MSH3 protein of *A. thaliana*. For instance, overexpression of MSH3 in a plant cell causes MSH2 present in the cell to be substantially completely complexed, disrupting the mismatch repair mechanism or mechanisms in the cell which are functionally dependent on the presence of a complex of MSH2 with MSH6. Similarly, mismatch repair mechanisms which depend on the presence of a complex of MSH2 and MSH3 may be disrupted by the overexpression of MSH6.

A chimeric gene of the fourth embodiment, incorporating a MMR altering gene, may be prepared by methods which are known in the art. Similarly, the MMR altering gene may be introduced into a plant cell, regenerating tissue or whole plant by techniques known in the art as being suitable for plant transformation, or by crossing. Known transformation techniques include *Agrobacterium tumefaciens* or *A. rhizogenes* mediated gene transfer, ballistic and chemical methods, and electroporation of protoplasts.

The MMR altering gene or genes are typically expressed from suitable promoters. Suitable promoters may direct constitutive expression, such as the 35S or the *NOS* promoter. Usually, however, the promoter will direct either inducible or tissue specific (e.g. callus; embryonic tissue; etc.), cell type specific (e.g. protoplasts; meiocytes; etc.) or developmental (e.g. embryo) expression of the altering gene or genes, in order for the

MMR system to function in tissue types or cell types, or at developmental stages of the plant, in which it is not desirable for the MMR system to be altered. Using such promoters, therefore, the activity of a MMR altering gene may be limited to a specific stage during plant development or it may be altered by controlling conditions external to the plant, and the deleterious effects of a permanently disabled or altered DNA mismatch repair system in a plant may be avoided. Examples of suitable promoters which are not constitutive are known in the art and include inducible promoters such as *PR1a* (reviewed by Gatz, 1997, Annual Rev. Plant Phys. Plant Mol. Biol. 48: 89), tissue specific promoters such as *AoPR1* (Sabahattin et al., 1993, Biotechnology 11: 218), and cell-type specific promoters such as *DMC1*.

A chimeric gene in accordance with the invention may further be physically linked to one or more selection markers such as genes which confer phenotypic traits such as herbicide resistance, antibiotic resistance or disease resistance, or which confer some other recognisable trait such as male sterility, male fertility, grain size, colour, growth rate, flowering time, ripening time, etc.

The process of the tenth embodiment of the invention provides, for example, a process for generating intraspecies genetic variation by altering the mismatch repair system in a plant cell, in regenerating plant tissue or in a whole plant. The plant cell, regenerating tissue or whole plant includes and expresses one or more MMR altering genes which are capable of altering mismatch repair in the plant cell, regenerating tissue or whole plant. Alteration of MMR may be achieved, for example, by inactivating the genes encoding plant MSH3 and/or plant MSH6. It is preferred to inactivate the plant MSH3 and MSH6 encoding genes at the same time and in the same plant cell, regenerating tissue or whole plant. Typically in this preferred form of the invention inactivation of either plant MSH3 or MSH6 alone is insufficient to substantially alter the plant's mismatch repair system and only when both MSH3 and MSH6 are inactivated simultaneously is the plant's mismatch repair system sufficiently altered to prevent the MMR system from recognising base pair mismatches, base insertions or deletions as a result of DNA replication errors, DNA damage, or oligonucleotide induced site-specific mutagenesis. However, in some applications of the invention, inactivation of only one gene may also be used to cause genomic instability or increase the efficiency of site-specific mutagenesis.

If desired, the MMR altering gene or genes may later be rendered non-functional or ineffective, or may be removed from the genome of the plant cell, regenerating tissue or whole plant in order to restore mismatch repair in the plant cell, regenerating tissue or whole plant. The MMR altering gene or genes may be inactivated by means of known gene inactivation tools, such as ribozymes, or may be removed from the genome using gene elimination systems known in the art, such as *CRE/LOX*. It is preferred to render two genes, whose gene products combine to incapacitate MMR, ineffective by separating

the altering genes through segregation. Therefore, in a preferred embodiment of the invention a first plant cell or plant is generated in which only plant *MSH3* is incapacitated, and a second plant cell or plant is generated in which only plant *MSH6* is incapacitated. The combination of both genomes, for example by crossing, then produces significant
5 MMR deficiency in those cells or plants which have inherited both altering genes. If the altering genes are expressed from unlinked loci, gene segregation restores MMR activity in the progeny of the cells or plants.

In a process of the ninth embodiment of this invention, homeologous recombination is enhanced between different genomes, chromosomes or genes in plant cells or plants by
10 altering MMR in said plant cells or plants. Such genomes, chromosomes or genes are characterised in that they originate from different plant families, genera, species, subspecies, plant varieties or lines. Hybrid plant cells or hybrid plants may be produced by crossing, by cell fusion or by other techniques known in the art. These plant cells or plants are further characterised by expressing one or more genes that are capable of
15 altering mismatch repair in the plant cell or plants.

In the process of the ninth embodiment, the homeologous recombination is typically for the purpose of introducing a desired characteristic in the hybrid plant. In this typical application of the process of the ninth embodiment, and in the process of the tenth embodiment the desired characteristic may be any characteristic which is of value to the
20 plant breeder. Examples of such characteristics are well known in the art and include altered composition or quality of leaf or seed derived storage products (e.g. oil, starch, protein), altered composition or quality of cell walls (e.g. decrease in lignin content), altered growth rate, prolonged flowering, increased plant yield or grain yield, altered plant morphology, resistance to pathogens, tolerance to or improved performance under
25 environmental stresses of various kinds, etc.

In a preferred form of the tenth embodiment, an MMR altering gene is co-introduced along with the homeologous genome, chromosome or gene of another plant cell or plant into an MMR proficient plant cell or MMR proficient plant to produce a hybrid plant cell or hybrid plant in which homeologous recombination can occur.
30 Suitably, the MMR proficient plant cell or MMR proficient plant may also include an MMR altering gene. For example a gene capable of inactivating plant *MSH3* may be co-introduced along with the homeologous genome, chromosome or gene of another plant cell or plant into an MMR proficient plant cell or MMR proficient plant in which *MSH6* is inactivated. A resultant hybrid plant in which homeologous recombination occurs will
35 include both the *MSH3* and *MSH6* altering genes and its MMR system will therefore be inactivated.

In this form of the invention, if hybrid plants are to be produced by crossing, the MMR altering gene preferably originates from the male parent, thus ensuring that the

MMR altering gene is always introduced and is not present in the recipient cell. That is, the MMR of the recipient cell, prior to introduction of the MMR altering gene, is typically proficient. Alternatively, if an MMR altering gene is present in a recipient cell it may be ineffective or inefficient on its own, or it may be linked to an inducible or tissue
 5 specific or cell type specific promoter which only renders the MMR altering gene active under limited conditions.

Thus, in a preferred form of the process of the ninth embodiment, the MMR system of the hybrid plant is initially unaltered. In this form of the process, the step of altering the mismatch repair system may comprise introducing into the hybrid plant, or cells
 10 thereof, a MMR altering gene, such as by *Agrobacterium tumefaciens* or *A. rhizogenes* mediated gene transfer, ballistic and chemical methods, and electroporation of protoplasts.

The MMR altering gene or genes are typically expressed from suitable promoters, as described above. Preferably, the promoter is transcriptionally active in mitotically and meiotically active tissue and/or cells to ensure MMR alteration after chromosome pairing
 15 at mitosis and meiosis, respectively. The preferred timing for MMR alteration is at meiosis, because recombinant genomes, chromosomes or genes are directly transmitted to the progeny. A suitable meiocyte specific promoter is for example the *DMC1* promoter from *Arabidopsis thaliana* ssp. *Ler.* (Klimyuk and Jones, 1997, Plant J. 11, 1-14). However, mitotic homeologous recombination is also a desirable outcome as somatic
 20 recombination events can be transmitted to offspring due to the totipotency of plant cells and the lack of predetermined germ cells in plants.

If desired, the MMR altering gene or genes may later be rendered non-functional or ineffective, or may be removed from the hybrid plant or hybrid plant cells, in order to restore mismatch repair in the hybrid plant or hybrid plant cells. The MMR altering gene
 25 or genes may be inactivated by means of known gene inactivation tools as described herein above.

EXAMPLES

Example 1. Cloning of the *AtMSH3* and *AtMSH6* coding sequences

Isolation of partial *AtMSH3* and *AtMSH6* consensus sequences

30 Degenerate oligonucleotides UPMU (SEQ ID NO:1) and DOMU (SEQ ID NO:2)

UPMU CTGGATCCACIGGICCAA(C/T)ATG

DOMU CTGGATCC(A/G)TA(A/G)TGIGTI(A/G)C(A/G)AA

were used to isolate *AtMSH3* and *AtMSH6* sequences by PCR amplification.

Primers UPMU and DOMU correspond to conserved amino acid sequences of the
 35 proteins MutS (*E. coli* and *S. typhimurium*), HexA (*S. pneumoniae*), Rep1 (mouse) and Dcl1 (human). The conserved regions to which they are targeted are TGPNM for UPMU (amino acid positions 852-856 for *AtMSH6* and 816-820 for *AtMSH3*) FATHY or FVTHY

for DOMU (amino acid positions 964-968 for AtMSH6 and 928-932 for AtMSH3, respectively.) These primers have been used to isolate MSH2 and MSH1 from yeast (Reenan and Kolodner, Genetics 132: 963-973 (1992)) and MSH2 from *Xenopus* and mouse (Varlet et al., Nuc. Acids Res. 22:5723-5728 (1994)).

5 Template single strand cDNA was produced by reverse transcription of 2 µg total RNA from a cell suspension culture of *Arabidopsis thaliana* ecotype Columbia (Axelos et al. 1989, Mol. Gen. Genetics 219: 106-112). The PCR reaction was performed under the following conditions in a final volume of 100µl: 0.2mM dNTP, 1µM each primer, 1XPCR buffer, 1u *Taq* DNA polymerase (Appligene) in the presence of template cDNA. PCR
10 parameters were 5 minutes at 94°C, followed by 30 cycles of 40 seconds at 95°C, 90 seconds at 45°C, 1 minute at 72°C. The amplification products were cloned into pGEM-T vector (Promega) and sequenced. Two different clones were isolated, S5 (350bp) was homologous to *MSH3*, S8 (327bp) was homologous to *MSH6*. Complete cDNA sequences were then isolated according to the Marathon cDNA amplification kit procedure (Clontech).
15 In summary, this procedure involves producing double stranded cDNA by reverse transcription of 2µg polyA+ RNA from the cell suspension culture of *Arabidopsis*. Adaptors are ligated on each side of the cDNA. The ligated cDNA is used as a template for 5' and 3' RACE PCR reactions in the presence of primers that are specific for the adaptor on one side (AP1 and AP2), and specific for the targeted gene on the other side. A 5' and a 3'
20 fragment that overlap are thus produced for each gene. The complete gene coding sequence can be reconstituted taking advantage of a unique restriction site, if available, in the overlapping region. Specific details of this procedure as it was used to isolate *AtMSH3* and *AtMSH6* coding regions, are as follows.

Isolation of *AtMSH3* complete coding sequence

25 From the sequence of clone S5, primer 636 (SEQ ID NO:3) was designed:

636 TGCTAGTGCCTCTTGCAAGCTCAT.

Primer AP1 (SEQ ID NO:4) is complementary to a portion of an adaptor sequence which had been ligated to the 5' and 3' ends of *Arabidopsis* cDNA:

AP1 CCATCCTAATACGACTCACTATAGGGC.

30 PCR performed on the ligated cDNA with primers 636 and AP1 for the 5' RACE PCR was followed by a second round of amplification with the nested primers AP2 (SEQ ID NO:5) and S525 (SEQ ID NO:6)

AP2 ACTCACTATAGGGCTCGAGCGGC

S525 AGGTTCTGATTATGTGTGACGCTTTACTTA

35 (the latter was also designed to correspond to a part of the sequence of clone S5) and produced a 2720bp DNA fragment. Figure 1 provides a diagrammatic representation of the primer sequences used to isolate *AtMSH3*. Another primer (S51, SEQ ID NO:7)

S51 GGATCGGGTACTGGGTTTTGAGTGTGAGG

was designed closer to the 5' border and permitted the determination of 99bp upstream to the ATG initiation codon. For the 3' RACE PCR, a first PCR reaction was performed with primers AP1 and 635 (SEQ ID NO:8).

635 GCACGTGCTTGATGGTGTTCAC

5 followed by a second round of amplification, using the nested primers AP2 and S523 (SEQ ID NO:9)

S523 TCAGACAGTATCCAGCATGGCAGAAGTA

which produced a DNA fragment of 890bp. Both DNA fragments were subcloned into pGEM-T and sequenced. Since PCR amplification using the Expand Long Template PCR
10 System (Boehringer-Mannheim) produced errors in the sequence, new oligonucleotides were designed to isolate those sequences again by PCR, but with the high fidelity DNA polymerase *Pfu*. PCR with primers 1S5 (SEQ ID NO:10) and S53 (SEQ ID NO:11)

1S5 ATCCCGGGATGGGCAAGCAAAAGCAGCAGACGA

S53 GACAAAGAGCGAAATGAGGCCCTTGG

15 amplified the 1244bp fragment clone 52 (SEQ ID NO:12, cloned into pUC18/*Sma*I). PCR with primers S52 (SEQ ID NO:13) and 2S5 (SEQ ID NO:14)

2S5 ATCCCGGGTCAAAATGAACAAGTTGGTTTTAGTC

S52 GCCACATCTGACTGTTCAAGCCCTCGC

amplified the 2104bp clone 13 (SEQ ID NO:15, cloned into pUC18/*Sma*I). The complete
20 coding sequence of the *AtMSH3* gene was reconstructed in pUC18 by ligating the 5' half of *AtMSH3* (clone 52) to the 3' half of *AtMSH3* (clone 13) after digesting with *Bam*HI which has a unique cleavage site in the overlapping region of both clones. This manipulation yielded plasmid pPF26. The *Sma*I fragment from pPF26 contains the complete *AtMSH3* coding sequence. The remaining primers referred to in Figure 1 are as
25 follows:

S51 GGATCGGGTACTGGGTTTTGAGTGTGAGG (SEQ ID NO:16)

S525 AGGTTCTGATTATGTGTGACGCTTTACTTA (SEQ ID NO:17)

Figures 2 and 3 provide plasmid maps of clones 52 and 13 respectively, showing restriction enzyme cleavage sites. The complete *AtMSH3* coding sequence (SEQ ID NO:18)
30 is 3246bp long and is shown in Figure 4 together with the deduced sequence (SEQ ID NO:19) of the encoded polypeptide. *AtMSH3* is clearly homologous to the yeast and mouse *MSH3* genes. A sequence alignment of polypeptides encoded by *AtMSH3* and that encoded by *Saccharomyces cerevisiae* *MSH3* is set out in Figure 5.

Isolation of the *AtMSH6* complete coding sequence and genomic sequences

35 The same procedure allowed isolation of the *AtMSH6* cDNA. Figure 6 provides a diagrammatic representation of the primer sequences used to isolate *AtMSH6*. For the 5' RACE PCR, primers 638 (SEQ ID NO:20) and AP1 (SEQ ID NO:4)

638 TCTCTACCAGGTGACGAAAACCG

allowed the amplification of a 2889 DNA fragment. Primer S81 (SEQ ID NO:21)

S81 CGTCGCCTTTAGCATCCCCTTCCTTCAC

helped define the 142bp upstream to the ATG initiation codon. On the 3' side, RACE PCR was initially performed with primers S823 (SEQ ID NO:22) and AP1 (SEQ ID NO:4),

S823 GCTTGGCGCATCTAATAGAATCATGACAGG

5 and then with the nested primers 637 (SEQ ID NO:23) and AP2 (SEQ ID NO:5).

637 GACAGCGTCAGTTCTTCAGAATGC

to produce a 774bp DNA fragment. As for *AtMSH3*, those fragments were cloned and sequenced. Re-isolation of the DNA sequence using the high fidelity *Pfu* polymerase and newly designed primers 1S8 (SEQ ID NO:24) and S83 (SEQ ID NO:25) (for the 5' side) led
10 to a 2182 bp DNA fragment identified as clone 43 (SEQ ID NO:26, cloned in pUC18/SmaI), and a 1379bp clone identified as clone 62 (SEQ ID NO:27, also cloned in pUC18/SmaI).

1S8 ATCCCGGGATGCAGCGCCAGAGATCGATTTTGT

2S8 ATCCCGGGTTATTTGGGAACACAGTAAGAGGATT (SEQ ID
15 NO:28)

S82 GCGTTCGATCATCAGCCTCTGTGTTGC (SEQ ID NO:29)

S83 CGCTATCTATGGCTGCTTCGAATTGAG

Figures 7 and 8 provide plasmid maps of clones 43 and 62 respectively, showing restriction enzyme cleavage sites. Clones 43 and 62 were digested by the *XmnI* restriction enzyme for
20 which a unique site is present in their overlapping region and then ligated. The complete *AtMSH6* coding sequence (SEQ ID NO:30) is 3330bp long and is shown in Figure 9 together with the deduced sequence (SEQ ID NO:31) of the encoded polypeptide. *AtMSH6* is clearly homologous to the yeast and mouse *MSH6* genes. A sequence alignment of polypeptides encoded by *AtMSH6* and that encoded by *Saccharomyces cerevisiae MSH6* is
25 set out in Figure 10.

An *AtMSH6* genomic sequence was also isolated from a genomic DNA library constituted after partial *Sau3AI* digestion of DNA from the *Arabidopsis* cell suspension. 8062bp were sequenced that covered the *AtMSH6* gene and show colinearity with the cDNA. 16 introns are found scattered along the gene. The complete genomic sequence
30 (SEQ ID NO:98) is shown in Figure 11.

Example 2. A measure of somatic variation in MMR deficient plants

Constructs

Constructs with antisense *AtMSH3* or antisense *AtMSH6* or both *AtMSH3/AtMSH6* under the control of a single 35S promoter have been inserted into the binary vector
35 pPZP121 (Hajdukiewicz et al., Plant Mol. Biol. 23, 793-799) between the right and left borders of the T-DNA. The pPZP121 plasmid confers chloramphenicol resistance to *Escherichia coli* or *Agrobacterium tumefaciens* bacteria. The *aacC1* gene is carried by the T-DNA and allows selection of transformed plant cells on gentamycin (Hajdukiewicz et al., Plant Mol. Biol. 25, 989-994). For the purpose of expressing antisense constructs, a 35S

promoter/terminator cassette with a polylinker was introduced into pPZP121. The 3' ends of the considered genes have been chosen since this region seems more efficient for antisense inhibition. For *AtMSH3* this corresponds to clone 13 (2104bp). for *AtMSH6* this corresponds to clone 62 (1379bp). Clone 13 comprises 2104bp of the 3' region that were cut off the pUC18 vector by *SalI/SstI* restriction, blunted with T4 DNA polymerase and ligated into the T4 DNA polymerase blunted *BamHI* site of pPZP121/35S, creating clone pPF13. The same procedure was followed for the 3' region of *AtMSH6* clone 62 (1379bp) thus creating plasmid pPF14. For the double constructs, the 3' region (from clone 62) of *AtMSH6* was introduced ahead of the *AtMSH3* region into pPF13 creating pCW186 and reciprocally, the 3' region of *AtMSH3* (from clone 13) was introduced ahead of *AtMSH6* into pPF14, creating pCW187.

These constructs were introduced into the Arabidopsis cells (as described below) of wildtype Columbia and of the Columbia tester line.

An alternative strategy to antisense inhibition of *AtMSH6* comes from experiments of Marra et al. (1998, Proc. Natl. Acad. Sci USA 95, 8568-8573) who show that overexpression of functional *MSH3* results in depletion of MSH6 protein in human cells. This depletion may generate a mismatch repair mutant phenotype.

For the purpose of overexpressing functional *AtMSH3* protein in plant cells, the complete *MSH3* coding region was excised from pPF26 (example 1) by digestion with *SmaI*, and was inserted into the *SmaI* site of pCW164. The resulting construct was named pPF66. It contains a complete *AtMSH3* gene under the control of the 35S promoter inside the left (LB) and right (RB) border of the T-DNA. This T-DNA also contains the *hpt2* gene for gentamycin selection. Plasmid pPF66 was introduced into Arabidopsis cells as described below. One cell clone was selected which clearly overexpressed the *AtMSH3* gene as shown by Northern analysis. Figures 12-16 provide plasmid maps of plasmids pPF13, pPF14, pCW186, pCW187 and pPF66, respectively.

Construction of tester construct

For the purpose of Forward Mutagenesis Assays, a tester construct was built containing the coding regions for *nptII*, *codA*, *uidA*. All three genes are driven by the 35S promoter and are terminated by the 35S terminator. This construct was obtained by introducing an *EcoRI* fragment encoding the *codA* cassette (2.5kb) and a *HindIII* fragment encoding the *uidA* (*GUS*) cassette (2.4kb) into the pPZP111 vector (Hajdukiewicz et al., 1994, Plant Mol Biol 23: 793-799) which already contained the *nptII* expression cassette. This new plasmid was named pPF57. *NptII* is used to select for transformed plant cells. *GUS* is used to analyse the degree of gene silencing in the construct (i.e. to identify cell lines in which the transgenes are expressed), and *codA* is used as a marker for forward mutagenesis (described below).

The plasmid map of pPF57 is provided in Figure 17.

Plant cell transformation

The constructs are introduced into *Agrobacterium* by electroporation. Plant cells are then transformed by co-cultivation. A suspension culture of *Arabidopsis thaliana* cells that has been established by Axelos et al. (1992, Plant Physiol. Biochem. 30, 1-6) may be used. One day old freshly subcultured cells are diluted five times into AT medium (Gamborg B5 medium, 30g/l sucrose, 200µg/l NAA). 10µl of saturated *Agrobacterium* containing the transforming T-DNA constructs are added to 10ml diluted cells in a 100ml erlenmeyer. The co-cultivation is agitated slowly (80rpm) for 2 days. The cells are then washed 3 times into AT medium and finally resuspended in the same initial volume (10ml). The culture is agitated for 3 days to allow expression before plating on selection plates (AT/BactoAgar 8g/l+gentamycin 50µg/ml). Transformed individual calli are isolated 3 weeks later.

Tester Strain

The tester construct on plasmid pPF57 was introduced into *Arabidopsis* cells of wildtype Columbia using the transformation protocol described above. Among 10 candidate transformants, one cell clone was shown (by Southern analysis) to have a unique T-DNA insertion. All three genes were shown to be functional in this cell line as indicated by resistance to kanamycin, blue staining in the presence of X-Glu (*GUS*), and sensitivity to 5-fluoro-cytosine (*codA*).

MMR altering genes (described above) were then introduced individually into the tester line and transformed cells are used for analysis of both Microsatellite Instability and Forward Mutagenesis.

Microsatellite analysis

Microsatellites have been described in *Arabidopsis* (Bell and Ecker, 1994, Genomics 19, 137-144). The present Example is based on a study of instability of microsatellites that are polymorphic amongst different ecotypes. DNA is extracted from the transformed calli. Specific primers have been defined that are used to amplify the microsatellite sequence. One of the two primers is previously P³² labelled by T4 kinase. In case of a polymorphic variation, new PCR products appear that do not follow the expected pattern of migration on a polyacrylamide gel. This is a commonly observed feature for MMR deficient cells in yeast or mammalian cells.

In particular, the present Example describes a study on microsatellites ca72 (CA₁₈), nga172 (GA₂₉), and ATHGENEA(A₃₉), chosen because they belong to the types predominantly affected in human mismatch repair deficient tumors. The size of these microsatellites is not conserved from one *Arabidopsis* ecotype to the other.

Arabidopsis cells which are transformed with an MMR altering gene (above) and control cells not expressing the MMR altering gene are allowed to form calli. DNA is

rapidly extracted from the calli and is analysed for microsatellite instability as described in detail by Bell and Ecker 1994, Genomics 19, 137-144. In summary, the relevant microsatellite is amplified by PCR using P32 labelled primers. The PCR products are separated on a DNA sequencing gel for size determination. Size differences between 5 microsatellites from transformed and control cells not expressing the MMR altering gene in question indicate microsatellite instability as a result of MMR alteration.

The sequences of primers used for PCR amplification of microsatellites ca72 and nga172 are included in Table 1. PCR amplification of microsatellite ATHGENEA made use of a forward primer containing the sequence

10 ACCATGCATAGCTTAACTTCTTG (SEQ ID NO:32)

and of a reverse primer containing the sequence

 ACATAACCACAAATAGGGGTGC (SEQ ID NO:33).

The amplification for microsatellite ca72 revealed in Columbia control cells (with respect to the MMR altering gene) a 248 bp long PCR fragment instead of the published 15 length of 124 bp. DNA sequencing verified this fragment as a CA₁₈ microsatellite.

Forward mutagenesis assay

Tester cells transformed with antisense *AtMSH3* or antisense *AtMSH6* or both *AtMSH3/AtMSH6* are analysed for the stability of the *codA* gene. The functional *codA* gene confers to sensitivity to 5-fluoro-cytosine (5FC), whereas a gene inactivating mutation in 20 *codA* will confer resistance to 5FC. The frequency of resistant cells is therefore a good indicator of somatic variation as a direct result of MMR alteration. Variants resistant to 5FC are first analysed for GUS activity. If GUS is inactive, 5FC resistance is assumed to be due to gene silencing (all three genes are under the 35S promoter). If GUS is active, 5FC resistance is assumed to be due to forward mutations that have inactivated *codA*. PCR is 25 then performed on the putative *codA* mutant genes which is then sequenced to confirm the presence of forward mutations in *codA*.

Besides *codA*, other marker genes may also be used for the Forward Mutagenesis Assay such as the *ALS* gene (conferring sensitivity to valine or to sulfonylurea; Hervieu and Vaucheret, 1996, Mol. Gen. Genet. 251 220-224; Mazur et al. 1987, Plant Physiol. 85 1110-30 1117).

Example 3. Homeologous meiotic recombination in *Arabidopsis thaliana*

A. Construction of a meiocyte specific gene expression cassette comprising the *DMC1* promoter and the *NOS* terminator

(i) The *DMC1* promoter may be used as published by Klimyuk and Jones, 1997, 35 Plant J. 11.1-14). To obtain a more convenient alternative for gene cloning, a 3.3 Kb

long subfragment of the *DMC1* promoter was obtained by PCR from genomic DNA of *Arabidopsis thaliana* (ssp. Landsberg erecta "Ler").

The PCR was done in three rounds:

Round One: A 3.7 Kb long product was obtained using the forward primer
5 DMCIN-A comprising the sequence

GAAGCGATATTGTTCGTG (SEQ ID NO:34)

and the reverse primer DMCIN-B comprising the sequence

AGATTGCGAGAACATTCC (SEQ ID NO:35).

The weak amplification product was then used as template for round two and three.

10 Round Two: A 3.1 Kb long product comprising the promoter and the 5' untranslated leader was obtained using forward primer DMCIN-1, which contained the sequence

acgcgtcgacTCAGCTATGAGATTACTCGTG (SEQ ID NO:36)

and introduced a *SalI* cloning site at the 5' end of the promoter fragment, and reverse
15 primer DMCIN-2 which contained the sequence

gctctagaTTTCTCGCTCTAAGACTCTCT (SEQ ID NO:37)

and introduced a *XbaI* site at the 3' end of the PCR fragment.

Round Three: A 0.2 Kb long product comprising the first exon/intron of the *DMC1* promoter was obtained using forward primer DMCIN-3, which contained the sequence

20 gctctagaGCTTCTCTTAAGTAAGTGATTGAT (SEQ ID NO:38)

and introduced a *XbaI* site at the 5' end of the PCR fragment, and reverse primer DMCIN-4, containing the sequence

tccccgggctcgagagatcctcatggTTTCTTCAGCTCTATGAATCC (SEQ ID NO:39)

and introduced at the 3' end of the PCR product restriction sites for *NcoI*, *BglII*, *XhoI* and
25 *SmaI*.

The products obtained in round Two and Three were digested with *XbaI* and subsequently ligated to reconstitute a 3.3 Kb long *DMC1* promoter from which the first two in-frame ATG start codons were replaced with a unique restriction site for *XbaI*. This promoter can be cloned between the restriction sites for *SalI* and *SmaI* of p3264, which contains the *SacI-EcoRI* NOS terminator in pBIN19, to yield the entire expression
30 cassette in pBIN19. This cassette contains the following cloning sites: *NcoI*, *BglII*, *XhoI*, *SmaI* and (already present on p3264) *KpnI* and *SacI*.

(ii) Another strategy yielded the following convenient *DMC1* promoter. A 1.8 kb DNA fragment comprising the 3' terminal part of the meiocyte specific *DMC1* promoter
35 was isolated by PCR from purified genomic DNA of *Arabidopsis thaliana* (ssp. Landsberg erecta "Ler"). The forward PCR primer (DMC1a) contained the sequence

acgcgtcgacGAATTCGCAAGTGGGG (SEQ ID NO:40)

and introduced a *SalI* cloning site at the 5' end of the promoter fragment. The reverse PCR primer (DMC1b) contained the sequence

tcacatggagatctcccggtacCGATTGCTTCGAGGG (SEQ ID NO:41)

introducing a polylinker region at the 3' end of the promoter fragment. The PCR reaction was carried out with VENT DNA Polymerase (NEB) over 25 cycles using the following cycling protocol: 1 minute at 94°C, 1 minute at 54°C, 2 minutes at 72°C.

5 The PCR reaction yielded a blunt ended DNA fragment which was digested with restriction enzyme *SaII* and was cloned into the cleavage sites of restriction enzymes *SaII* and *SmaI* in plasmid p2030, a pUC118 derivative containing the *SacI-EcoRI* NOS terminator fragment of pBIN121. The cloning yielded plasmid p2031, containing the *DMC1* promoter-polylinker-NOS terminator expression cassette depicted in Figure 18.

10 B. Construction of an *MSH3* antisense gene under the control of the *DMC1* promoter

A 2.1 kb DNA fragment encoding the carboxyterminal part of AtMSH3 was removed from the polylinker of clone 13 described in Example 1 after (i) digestion with *KpnI*, (ii) blunting of the DNA ends generated by *KpnI* and (iii) digestion with *BamHI*. The isolated fragment was then cloned in antisense orientation downstream of the *DMC1*
15 promoter in plasmid p2031, which had been digested with restriction enzymes *SmaI* and *BglII*. This cloning yielded plasmid p2033 (Figure 18).

After digestion of p2033 with *EcoRI*, a 4.1 kb DNA fragment was recovered comprising the *DMC1* promoter, the partial *MSH3* cDNA in antisense orientation with respect to the promoter and the *NOS* terminator. This fragment was cloned into the *EcoRI*
20 restriction site of plant transformation vector pNOS-Hyg-SCV to yield plasmid p3242 (Figure 18).

C. Construction of a combined *MSH6/MSH3* antisense gene under the control of a single *DMC1* promoter

A 3.1 kb fragment, encoding in antisense orientation the partial AtMSH6 and AtMSH3
25 sequences and the 35S terminator, was isolated from pCW186 by digestion with *KpnI*. The fragment was treated with *Klenow* enzyme to blunt both ends. It was then cloned into the *SmaI* site of plasmid p3243 (a pNOS-Hyg-SCV derivative, illustrated in Figure 19), which had been opened to delete the region between the *SmaI* sites. Clones containing the fragment in the antisense orientation with respect to the *DMC1* promoter (described in
30 A(ii) above) were identified by diagnostic digestion with *BamHI*. The selected construct was named p3261.

Another practical way of cloning the double antisense gene is as follows. A 1 kb DNA fragment encoding the carboxyterminal part of AtMSH6 is isolated from clone 62 described in Example 1 after digestion of clone 62 plasmid DNA with *BamHI*, which
35 cleaves in the 5' polylinker region flanking the partial cDNA, and with *EcoRI*, which cleaves within the cDNA. The isolated fragment is treated with *Klenow* enzyme to blunt both its ends and is cloned into the recipient plasmid p2033 or p3242. For the purpose of

cloning, the recipient plasmid may be cleaved with either *AvaI* or *NcoI* and can be blunted with *Klenow* enzyme to produce blunt acceptor ends for fragment cloning. This cloning yields two opposite orientations of cloned fragment DNA with respect to the *DMC1* promoter. These can be identified by diagnostic digestion with restriction enzymes *ScaI* or *XmnI* in conjunction with *SacI*. The selected construct contains the *DMC1* promoter, the combined partial cDNAs for *AtMSH3* and *AtMSH6* (both cloned in antisense orientation with respect to the *DMC1* promoter) and the *NOS* terminator. If the recipient plasmid is p2033, the combined antisense gene under control the single *DMC1* promoter is recovered from the vector after *EcoRI* digestion and is cloned into the *EcoRI* restriction site of pNOS-Hyg-SCV.

D. Construction of a full-length *MSH3* sense gene under control of the *DMC1* promoter for overexpression of functional *MSH3* protein

Overexpression of *MSH3* protein was shown in human cells (Marra et al., 1998, Proc. Natl. Acad. Sci. USA 95, 8568-8573) to complex all available *MSH2* protein. This leaves *MSH6* protein without its partner, leading to the degradation of *MSH6* protein and eventually to a mismatch repair phenotype.

This phenomenon is exploited to increase homeologous meiotic recombination in *Arabidopsis* as an alternative to antisense inhibition of *AtMSH6*. For this purpose the full-length cDNA encoding *AtMSH3* is isolated from plasmid pPF66 by digestion with *SmaI* and is cloned into the *SmaI* site of the *DMC1* expression cassettes described in A(i).

E. Selection of Recombination markers on homeologous chromosomes of *Arabidopsis thaliana* subspecies *Landsberg erecta* (Ler), *Columbia* (Col) and *C24*, respectively

E(i). Visual recombination markers in *Arabidopsis th.* subspecies *C24*:

Agrobacterium mediated transformation with a T-DNA containing a *35S-GUS* gene, inactivated by insertion of a *35S-Ac* transposable element (Finnegan et al., 1993, Plant Mol. Biol. 22, 625-633), had yielded a *C24* line in which the T-DNA construct was integrated into chromosome 2. Genetic and molecular analysis of this line shows that the *Ac* transposon had excised from its T-DNA locus thereby restoring *GUS* activity, but had re-inserted into the chromosome at a distance of 16.4 cM, where it stayed fixed (due to disablement of *Ac*) within the *chlorina* gene. Insertional inactivation of the *chlorina* gene caused a bleached phenotype in those plants that were homozygous for this mutation. Because of the two linked phenotypic markers, *chlorina3A:Ac* and *GUS*, this *C24* line was used in crosses to wildtype Ler for analysis of meiotic homeologous recombination on chromosome 2 in conjunction with molecular recombination markers.

E(ii). Visual recombination markers in *Arabidopsis th.* *Ler*:

The Ler line NW1 (obtained from NASC, Nottingham, UK) contains one recessive visual marker per chromosome. i.e. *an-1* on Chr.1, *py-1* on Chr.2, *gll-1* on Chr.3, *cer2-1*

on Chr.4, and *msl-1* on Chr.5. This line is used in crosses to wildtype C24 which expresses an MMR altering gene for analysis of meiotic homeologous recombination on chromosomes 1-5 in conjunction with molecular recombination markers listed in Table 1.

Other *Ler* lines from NASC have several visual markers in close proximity to each other on the same chromosome. When these lines are used for hybrid production, analysis of homeologous meiotic recombination may make use entirely of visual recombination markers. Particularly suitable for crossing to C24 wildtype that is expressing a MMR altering gene are the following *Ler* lines:

NW22: relative markers are *dis1* - (4 cM) - *ga4* - (11 cM) - *th1* on chromosome 1.

10 NW10: relevant markers are *tz-201* - (5 cM) - *cer3* on chromosome 5.

NW134, relevant markers are *ttg* - (4 cM) - *ga3* on chromosome 5.

NW24 (*abi3-1*) and NW64 (*gll-1*). When present in the same plant on chromosome 3, *abi3-1* and *gll-1* are 13 cM apart. Since this marker combination is not available from NASC, we have combined these markers by crossing line NW24 to line NW64. The F1
15 offspring were allowed to self-fertilise and to produce F2 seeds. F2 Plants which carry both markers as homozygous traits on the same chromosome can be identified firstly, by germinating F2 seeds on germination medium containing selective concentrations of abscisic acid, and subsequently, by identifying among the abscisic acid resistant plants those individuals which show the glabra phenotype.

20 E(iii) Molecular recombination markers in *Col*, *Ler* and C24:

The genome of *Arabidopsis thaliana* is interspersed with unique base sequences arranged as simple tandem repeats. Allelic repeats can vary in length between different *Arabidopsis* subspecies and when amplified by PCR yield diagnostic DNA products of different length named Simple Sequence Length Polymorphisms (SSLPs). Many SSLPs
25 have been genetically mapped and have been assigned to unique chromosome locations on the recombinant inbred map (Bell and Ecker, 1994, Genomics 19, 137-144; Lister and Deans lines, Weeds World 4i, May 1997).

In Table 1 are listed 28 mapped and established SSLPs between *Ler* and *Col*. A number of PCR primer pairs are described herein (SEQ ID NO:42 to SEQ ID NO:97)
30 which also yielded SSLPs between C24 and *Ler* (19 SSLPs) or between C24 and *Col* (25 SSLPs), respectively. Polymorphic SSLPs can be used as molecular markers in the analysis of homeologous recombination between genomes from these subspecies.

The PCR reactions (25 µL) were carried out over 35 cycles (15 seconds at 94°C, 30 seconds at 55°C and 30 seconds at 72°C), with 0.25 U Taq DNA polymerase and 0.6 µg
35 genomic DNA in reaction buffer containing 2 mM MgCl₂. PCR products were separated by agarose gel electrophoresis (4% ultra high resolution agarose) and visualised by ethidiumbromide staining. The results from the PCR experiments are summarised in

Table 1, which also shows the sequence of PCR primers, primer annealing temperature (T_m), PCR product length and chromosome location of SSLP (with respect to the RI map of May 1997, Weeds World 4i).

F. Production of hybrid plants

- 5 C24 plants heterozygous for *chlorina3A:Ac/GUS* are crossed as male to emasculated wildtype *Ler* to produce *Ler/C24(chlorina3A, GUS)* hybrid seeds.

Due to the heterozygosity of the C24 parent, only 50 % of hybrid plants have inherited the *chlorina3A:Ac/GUS* locus. The remaining 50% of hybrid plants are wildtype with respect to *chlorina3A:Ac/GUS*. Since the mutant locus is linked to a kanamycin
10 resistance gene (contained on the same T-DNA as *GUS*) mutant plants can be pre-selected by germinating hybrid seeds on germination medium containing 50 mg/L kanamycin.

Ler plants homozygous for the five chromosome markers are male sterile (*msl-1*) and are crossed without emasculation to wildtype C24 to produce *Ler(an-1, py-1, gl1-1, cer2-1, msl-1)/C24* hybrid seeds.

- 15 Other *Ler* plants, which are male fertile, are crossed after emasculation of the female parent to produce *Ler/C24* hybrid seeds.

G. Introduction of *MSH3* and *MSH6/3* antisense genes into *Arabidopsis* and analysis of meiotic homeologous recombination

(i) Transformation of hybrid plants and analysis of homeologous meiotic recombination

- 20 The plant transformation vectors comprising the antisense genes described in (B) and (C) above are introduced into *Agrobacterium tumefaciens* strain AGL1 (Lazo et al., 1991, Bio/Technology 9, 963-967) by electroporation. Recombinant *Agrobacterium* clones are selected on LB medium containing 50 mg/L rifampicin and 100 mg/L carbenicillin. Selected clones are used to infect roots of *Arabidopsis* hybrid plants (described in (F)
25 above) using the root transformation protocol of Valvekens et al. (1988, PNAS 85, 5536-5540) except that shoot and root inducing media contain hygromycin (10 mg/L) instead of kanamycin.

Plants regenerated from roots of hybrid plants are genetic clones of root donating plants and therefore are again genetic hybrids of two *Arabidopsis* subspecies described in
30 (F). However, in contrast to the root donating plants, the regenerated hybrid plants also contain the introduced transgene and the co-introduced hygromycin resistance gene with the latter allowing these plants to grow on hygromycin containing culture medium.

Hygromycin resistant plants are then allowed to enter the reproductive phase and to produce gametes by meiotic divisions of microspore and megaspore mothercells. At
35 meiosis, the *DMC1* promoter is activated and can direct the expression of antisense genes described in (B) and (C) above, leading to decreased *MSH6* and/or *MSH3* gene

expression. This in turn depletes the gamete mothercells of MSH6 and/or MSH3 protein, thus causing alteration of MMR during meiotic divisions and increasing the recombination frequency between homeologous chromosomes.

Transgenic plants are then allowed to self-fertilise and to produce seeds. These
5 seeds (F2 seeds with respect to hybrid production), and the plants derived therefrom, carry the homeologous recombination events which can be identified by using the visual and molecular recombination markers described in (E) above.

In case of homeologous recombination between chromosomes of *Ler* and *C24(chlorina3A:Ac, GUS)*, the analysis concentrates on chromosome 2 by selecting plants
10 showing the visual phenotypic marker *chlorina*. This marker thus serves as a reference point as it indicates that respective chromosomes 2 originate from *C24*. Other markers, such as *GUS* or molecular markers, on chromosome 2 may then be used to identify chromosomal regions which are derived from the *Ler* chromosome as a result of homeologous recombination. F2 plants of control transformants not expressing the
15 antisense gene(s) can be analysed in parallel and the results can be used for comparison to homeologous recombination results obtained in antisense plants.

(ii) Transformation of C24 wildtype, hybrid plant production and analysis of homeologous meiotic recombination

Introduction of MMR altering genes into wildtype *C24* is done using the root
20 transformation protocol as described in G(i) for transformation of hybrid plants. Transformed plants are selected by resistance to either 10 mg/L hygromycin (in case of transformation with T-DNA's derived from pNOS-Hyg-SCV) or to 50 mg/L kanamycin (in case of transformation with T-DNA's derived from pBIN19).

Transgenic plants are then allowed to self-fertilise and to produce seeds (T1 seeds).
25 Segregation of the antibiotic resistance gene in the T1 population then indicates the number of transgene loci. Lines with a single transgene locus (indicated by a 3:1 ratio of resistant:sensitive plants) are selected and are bred to homozygosity. This is done by collecting selfed seeds (T2) from T1 plants and subsequent testing of at least four independent T2 seed populations for segregation of the antibiotic resistance gene. T2
30 populations which do not segregate the antibiotic resistance gene are assumed to be homozygous for both the resistance gene and the linked MMR altering gene.

C24 plants homozygous for the MMR altering gene are then crossed to *Ler* lines homozygous for recessive visual markers (see E(ii)) to produce *C24/Ler* hybrid plants as described in (F). These F1 hybrids are then allowed to enter the reproductive phase and to
35 produce gametes by meiotic division of microspore and megaspore mothercells. At meiosis, the *DMC 1* promoter is activated and can direct the expression of antisense or sense genes described in (B), (C) and (D) above, leading to decreased *MSH6* and/or *MSH3* gene expression. This in turn depletes the gamete mothercells of *MSH6* and/or *MSH3*

protein, thus causing alteration of MMR during meiotic divisions and increasing the recombination frequency between the homeologous chromosomes of *C24* and *Ler*. Recombination events are then scored in the F2 generation.

For recombination analysis, the hybrid plants are allowed to self-fertilise and to
5 produce F2 seeds. F2 plants are then preselected for a first visual marker. Since this marker is recessive, its visual presence indicates homozygosity for *Ler* DNA at the relevant locus. Those F2 plants which show this first visual marker are then analysed for the presence or absence of a second visual marker which in the *Ler* parent is closely linked to the first marker. Absence of the second visual marker indicates recombination between
10 the relevant *C24* and *Ler* chromosomes between the first and second marker. The frequency of recombination in transgenic hybrids is compared to the recombination frequency in control hybrids not expressing the MMR altering gene.

Examples of recombination analysis are the following.

The *Ler* line NW22(*dis1*, *ga4*, *th1*) is used for crosses to transformed *C24*.

15 F2 plants are preselected first for thiamine requirement (*th1*) and then are further analysed for re-appearance of wildtype height (loss of *ga4*) and/or re-appearance of normal trichomes (loss of *dis1*) as a result of recombination.

The *Ler* line NW10(*tz-201*, *cer3*) is used for crosses to transformed *C24*.

F2 plants are then preselected first for thiazole requirement (*tz*) and then are further
20 analysed for re-appearance of normal, i.e. non-shiny stems (loss of *cer3*) as a result of recombination.

The *Ler* line NW134 (*ttg*, *ga3*) is used for crosses to transformed *C24*. F2 plants are first preselected for dwarfish appearance (*ga3*) and are then analysed for re-appearance of trichomes (loss of *ttg*) as a result of recombination.

25 *Ler* plants homozygous for *abi3-1* and *gll-1* are used for crosses to transformed *C24*. F2 plants are first preselected for their ability to germinate in the presence of abscisic acid and are then analysed for re-appearance of trichomes on the leaves (loss of *gll-1*) as a result of recombination.

In the case of homeologous recombination between transformed *C24* and the *Ler* line
30 NW1 (*an-1*, *py-1*, *gll-1*, *cer2-1*, *msl-1*), recombination analysis is similar the one described above, except that the second marker is not a visual marker but has to be a molecular marker. This is because the *Ler* parent carries only one visual marker per chromosome.

TABLE 1: SSLP Markers in *Arabidopsis thaliana* Subspecies

Chromosome	RI Map Position	PCR Primer Pair	Primer Sequence	T _m [°C]	length/COL	length/LER	length/C24
I	2.3	ATEAT1 F	GCCACTGCGTGAATGATATG	57.8	172	162	162
		ATEAT1 R	CGAACAGCCCAACATTAATCC	58.2			
I	9.3	NGA63 F	AACCAAGGCACAGAAAGCG	57.3	111	89	120
		NGA63 R	ACCAAGTGATCGCCACC	59.6			
I	40.1	NGA248 F	TACCGAACCAAAACACAAAGG	56.1	143	129	no amplific.
		NGA248 R	TCTGTATCTCGGTGAATTCTCC	58.2			
I	81.2	NGA128 F	GGTCTGTTGATGTCGTAAAGTCG	60.1	180	190	no amplific.
		NGA128 R	ATCTTGAAACCTTTAGGGAGGG	58.2			
I	81.2	NGA280 F	CTGATCTCACGGACAAATAGTGC	60.1	105	85	85
		NGA280 R	GGCTCCATAAAAAAGTGCACC	57.8			
I	111.4	NGA111 F	CTCCAGTTGGAAGCTAAAGGG	60	128	162	170
		NGA111 R	TGTTTTTTAGGACAAATGGCG	70			
II	ca. 7.5	NGA168 F	CCTTCACATCCAAAACCCAC	57.8	213	217	208
		NGA168 R	GCACATACCCCAACACCAGAA	57.8			

II	ca. 48	NGA1126L	CGCTACGCTTTTCGGTAAAG	57.8	191	199	196
		NGA1126R	GCACAGTCCAAGTCACAACC	59.9			
II	62.2	NGA361L	AAAGAGATGAGAATTGGAC	51.7	114	120	114
		NGA361R	ACATATCAATATATTAAAGTAGC	49.5			
II	73	NGA168 F	TCGTCTACTGCACCTGCCG	59.6	151	135	135
		NGA168 R	GAGGACATGTATAGGAGCCTCG	61.9			
II	ca. 77	AthBIO2 L	TGACCTCCTCTTCCATGGAG	59.9	141	209	139
		AthBIO2 R	TTAACAGAAACCCAAAGCTTTC	54.5			
II	ca. 83	AthUBIQUE L	AGGCAATGTCCATTTCATTG	54.1	146	148	148
		AthUBIQUE R	ACGACATGGCAGATTTCCTCC	57.8			
III	3.4	NGA172 F	AGCTGCTTCCTTATAGCGTCC	60	162	136	140
		NGA172 R	CATCCGAATGCCATTGTTC	55.4			
III	12.8	NGA126 F	GAAAAACGCTACTTTCGTGG	56.1	119	147	no amplif.
		NGA126 R	CAAGAGCAATATCAAGAGCAGC	58.2			
III	17.5	NGA162 F	CATGCAATTTCATCTGAGG	55.8	107	89	no amplif.
		NGA162 R	CTCTGTCACCTCTTTCCTCTGG	60.1			

III	81.8	NGA6 F	TGGAATTTCTTCCTCTCTCTC	56.1	143	123	143
		NGA6 R	ATGGAGAAGCTTACACTGATC	56.1			
IV	19.8	NGA12 F	AATGTTGTCCTCCCTCCTC	59.9	247	234	220
		NGA12 R	TGATGCTCTCTGAACAAGAGC	58.2			
IV	24.1	NGA8 F	GAGGGCAAATCTTTATTTTCGG	56.1	154	198	190
		NGA8 R	TGGCTTTCGTTTATAAACATCC	54.5			
IV	102	NGA1107 L	GCGAAAAAACAACAAAAATCCA	50.2	150	140	140
		NGA1107 R	CGACGAATCGACAGAAATTAGG	58			
V	11.8	NGA225 F	GAAATCCAAATCCAGAGAGG	58	119	189	119
		NGA225 R	TCTCCCCACTAGTTTGTGTCC	60.1			
V	16.7	NGA249 F	TACCGTCAATTTTCATCGCC	55.4	125	115	115
		NGA249 R	GGATCCCTAACTGTAAAAATCCC	58.2			
V	19.9	CA72 F	AATCCCAGTAACCAACACACA	56.3	124	110	110
		CA72 R	CCCAGTCTAACCAACGACCAC	61.9			
V	20	NGA151 F	GTTTGGGAAGTTTGTCTGG	55.8	150	120	130
		NGA151 R	CAGTCTAAAGCGAGAGTATGATG	58.6			

V	24	NGA106 F	GTATGGAGTTCCTAGGGCAG	60.1	157	123	130
		NGA106 R	TGCCCCATTTGTCTCTC	55.8			
V	37.8	NGA139 F	AGAGCTACCAGATCCGATGG	59.9	174	132	132
		NGA139 R	GGTTTCGTTTCACTATCCAGG	55.8			
V	50	NGA76 F	GGAGAAAAATGTCACCTCTCCACC	60.1	231	> 250	300
		NGA76 R	AGGCATGGGAGACATTACG	57.8			
V	61.1	ATHSO191 L	CTCCACCAATCATGCAAATG	55.8	148	156	146
		ATHSO191 R	TGATGTTGATGGAGATGGTCA	53.7			
V	81.7	NGA129 F	TCAGGAGGAACTAAAGTGAGGG	60.1	177	179	172
		NGA129 R	CACACTGAAGATGGTCTTGAGG	60.1			

CLAIMS

1. An isolated and purified DNA molecule comprising a polynucleotide sequence encoding a polypeptide functionally involved in the DNA mismatch repair system of a plant.
- 5 2. A DNA molecule according to claim 1 wherein said polypeptide is homologous to a mismatch repair polypeptide of a yeast or of a human.
3. A DNA molecule according to claim 1 wherein said polypeptide is homologous to AtMSH3 (SEQ ID NO: 19) or to AtMSH6 (SEQ ID NO: 31).
4. An isolated and purified polypeptide functionally involved in the DNA
10 mismatch repair system of a plant.
5. A polypeptide according to claim 4 which is homologous to a mismatch repair polypeptide of a yeast or of a human.
6. An isolated and purified polypeptide selected from the group consisting of a polypeptide encoded by the gene *AtMSH3* (SEQ ID NO: 18), a polypeptide encoded by the
15 gene *AtMSH6* (SEQ ID NO:30), polypeptides homologous to a polypeptide encoded by the gene *AtMSH3* (SEQ ID NO: 18) and polypeptides homologous to a polypeptide encoded by the gene *AtMSH6* (SEQ ID NO:30).
7. An isolated and purified DNA molecule comprising a polynucleotide sequence selected from the group consisting of (i) a sequence encoding a polynucleotide which is
20 capable of interfering with the expression of a plant polynucleotide sequence encoding a polypeptide which is homologous to a mismatch repair polypeptide of a yeast or of a human and thereby disabling said plant polynucleotide sequence; and (ii) a sequence encoding a polypeptide capable of disrupting the DNA mismatch repair system of a plant.
8. A DNA molecule according to claim 7 comprising a polynucleotide sequence
25 encoding a polynucleotide capable of interfering with the expression of a plant polynucleotide sequence encoding a polypeptide which is homologous to a mismatch repair polypeptide of a yeast or of a human and thereby disabling said plant polynucleotide sequence.
9. A DNA molecule according to claim 8 wherein said polynucleotide is capable
30 of interfering with the expression of a plant polynucleotide sequence is a sense polynucleotide, an antisense polynucleotide or a ribozyme.
10. A DNA molecule according to claim 7 comprising a polynucleotide sequence encoding a polypeptide capable of disrupting the DNA mismatch repair system of a plant.

11. A DNA molecule according to claim 10 wherein said polypeptide is homologous to AtMSH3 (SEQ ID NO: 19) or to AtMSH6 (SEQ ID NO: 31).

12. A DNA molecule according to claim 10 further comprising a regulation element capable of causing overexpression of said polypeptide in a cell of said plant.

5 13. A chimeric gene comprising:

a DNA sequence selected from the group consisting of (i) a sequence encoding a polynucleotide capable of interfering with the expression of a plant polynucleotide sequence encoding a polypeptide which is homologous to a mismatch repair polypeptide of a yeast or of a human and thereby disabling said plant polynucleotide sequence, and (ii) a
10 sequence encoding a polypeptide capable of disrupting the DNA mismatch repair system of a plant; and

at least one regulation element capable of functioning in a plant cell.

14. A chimeric gene according to claim 13 wherein said regulation element is selected from constitutive, inducible, tissue type specific and cell type specific promoters.

15 15. A chimeric gene according to claim 13 comprising a DNA sequence encoding a polypeptide capable of disrupting the DNA mismatch repair system of a plant, wherein said regulation element is capable of causing overexpression of said polypeptide in a cell of said plant.

16. A chimeric gene according to claim 13 wherein said regulation element is
20 selected from the group consisting of 35S, NOS, PR1a, AoPR1 and DMC1.

17. A plasmid or vector comprising a chimeric gene according to any one of claims 13-16.

18. A plant cell stably transformed, transfected or electroporated with a plasmid or vector according to claim 17.

25 19. A plant comprising a cell according to claim 18.

20. A plant according to claim 19 selected from plants of the families *Brassicaceae*, *Poaceae*, *Solanaceae*, *Asteraceae*, *Malvaceae*, *Fabaceae*, *Linaceae*, *Canabinaceae*, *Dauaceae* and *Cucurbitaceae*.

21. A process for at least partially inactivating a DNA mismatch repair system of a
30 plant cell, comprising transforming or transfecting said plant cell with a DNA molecule according to any one of claims 1-3 or 7-12 and causing said DNA sequence to express said polynucleotide or said polypeptide.

22. A process for at least partially inactivating a DNA mismatch repair system of a plant cell, comprising transforming or transfecting said plant cell with a chimeric gene

according to any one of claims 13-16 and causing said DNA sequence to express said polynucleotide or said polypeptide.

23. A process for at least partially inactivating a DNA mismatch repair system of a plant cell, comprising transforming or transfecting said plant cell with a plasmid or vector
5 according to claim 17 and causing said DNA sequence to express said polynucleotide or said polypeptide.

24. A process for increasing genetic variation in a plant comprising obtaining a hybrid plant from a first plant and a second plant, or cells thereof, said first and second plants being genetically different; altering the mismatch repair system in said hybrid plant;
10 permitting said hybrid plant to self-fertilise and produce offspring plants; and screening said offspring plants for plants in which homeologous recombination has occurred.

25. A process according to claim 24 wherein a first gene is incapacitated in said first plant, a second gene is incapacitated in said second plant, and said first and second genes are incapacitated in said hybrid plant thereby altering the mismatch repair system of
15 said hybrid plant.

25. A process according to claim 25 wherein said incapacitation of the mismatch repair system of said hybrid plant is reversible.

26. A process according to claim 24 wherein a new genetic linkage of a desired characteristic trait or of a gene which contributes to a desired characteristic trait is
20 observable in at least one of said offspring plants.

27. A process for obtaining a plant having a desired characteristic, comprising altering the mismatch repair system in a plant, cell or plurality of cells of a plant which does not have said desired characteristic, permitting mutations to persist in said cells to produce mutated plant cells, deriving plants from said mutated plant cells, and screening
25 said plants for a plant having said desired characteristic.

28. A process according to claim 27 wherein said step of altering the mismatch repair system comprises introducing into said hybrid plant, plant, cell or cells a chimeric gene according to claim 13 and permitting the chimeric gene to express a polynucleotide which is capable of interfering with the expression of a plant polynucleotide sequence in a
30 mismatch repair gene of the hybrid plant, plant, cell or cells, or a polypeptide capable of disrupting the DNA mismatch repair system of the hybrid plant, cell or cells.

29. A process according to claim 28 comprising inactivating an MSH3 gene and/or an MSH6 gene of said plant.

30. A process according to claim 28 comprising inactivating an MSH3 gene and an
35 MSH6 gene of said plant.

31. A process according to claim 27 comprising at least partially inactivating the mismatch repair system of said plant in a predetermined cell type or in a predetermined tissue of said plant.

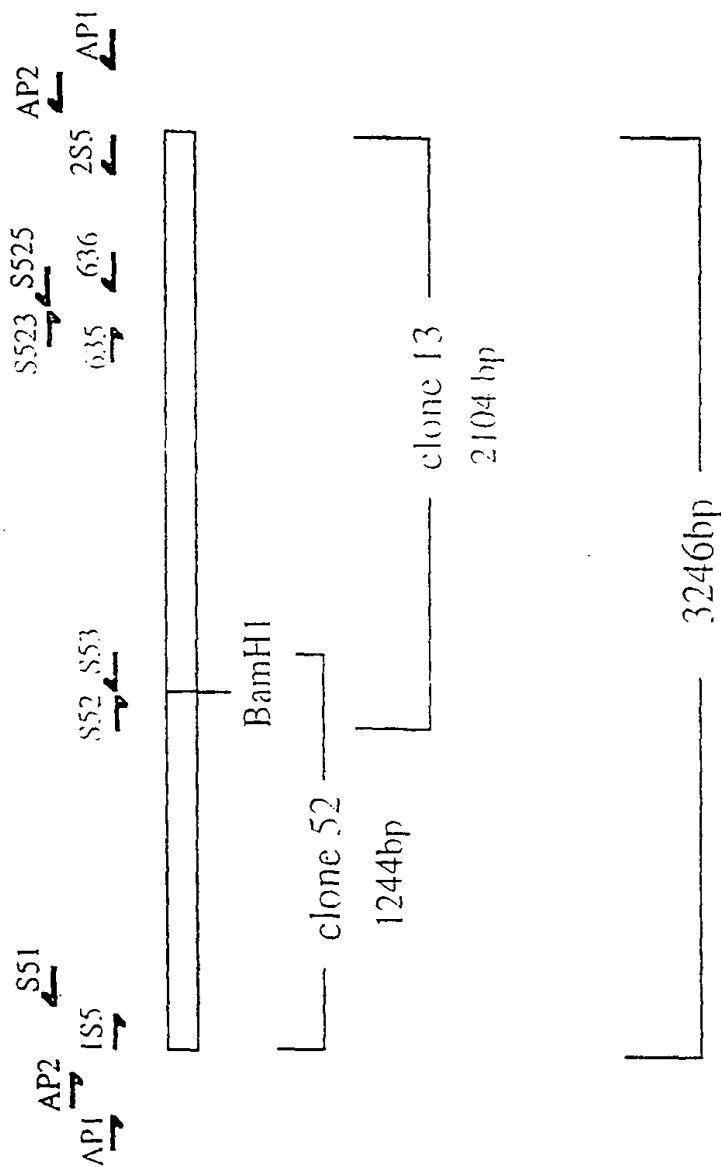
32. A process according to claim 31 further comprising restoring mismatch repair
5 in said cell type or said tissue.

33. An oligonucleotide capable of hybridising at 45°C under standard PCR conditions to a DNA molecule according to claim 1 with the proviso that said oligonucleotide is other than SEQ ID NO:1 or SEQ ID NO:2.

34. An oligonucleotide capable of hybridising at 45°C under standard PCR
10 conditions to the DNA of SEQ ID NO: 18 with the proviso that said oligonucleotide is other than SEQ ID NO:1 or SEQ ID NO:2.

35. An oligonucleotide capable of hybridising at 45°C under standard PCR conditions to the DNA of SEQ ID NO:30 with the proviso that said oligonucleotide is other than SEQ ID NO:1 or SEQ ID NO:2.

Figure 1



422 Rec'd PCT/PTO 1 0 APR 2000

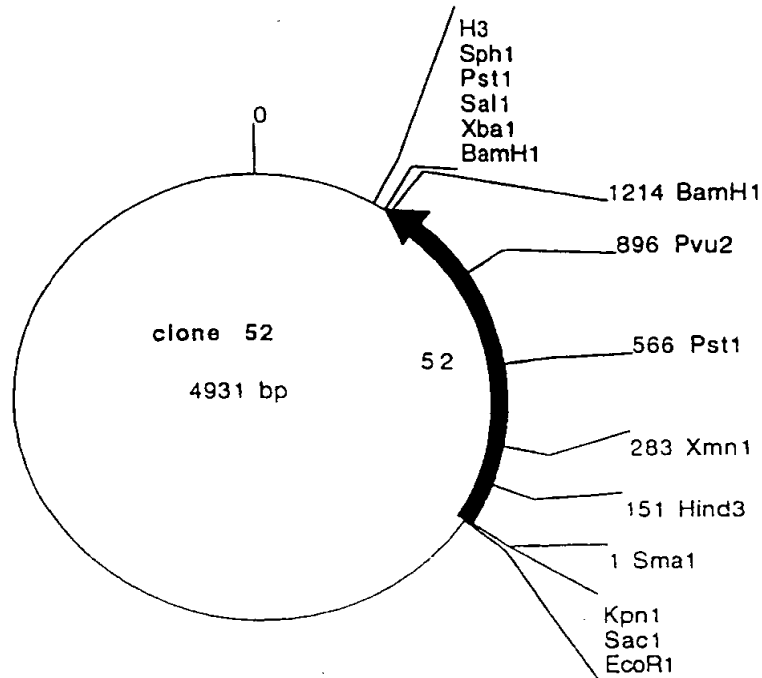


Figure 2

Comments/References: 52= 3' side of S5 (AIMSH3) 1244bp in pUC18/Sma1

422 Rec'd [REDACTED] PTO 1 0 APR 2000

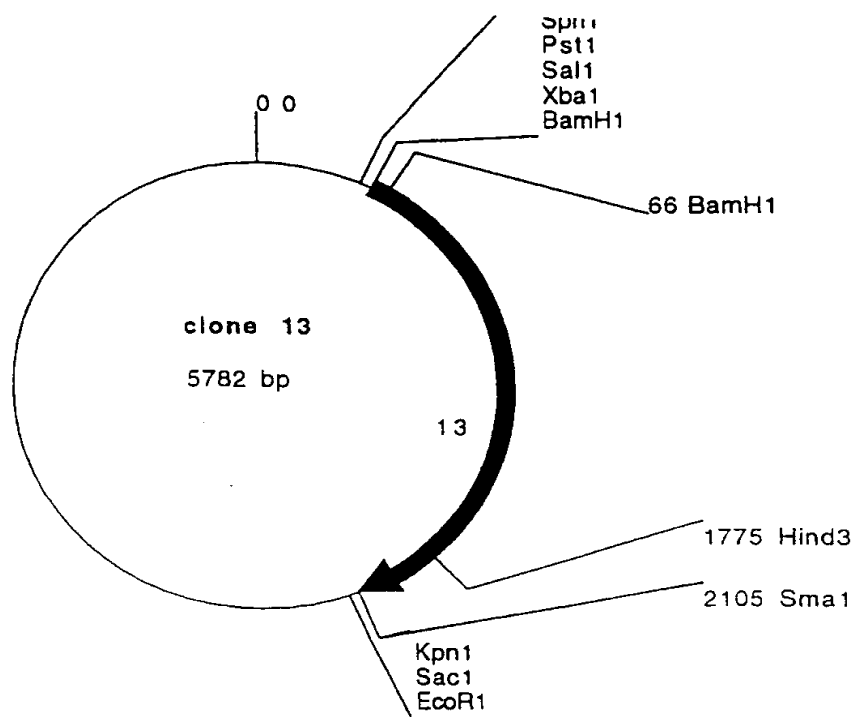


Figure 3

Comments/References: 13 = 3' side of S5 (AtMSH3) 2104bp in pUC18/Sma1

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1      CCTAAGAAAGCGCGGAAATTTGGCAACCAAGTTTGGCATAGCCAGACACCTTCCATTTCTCTTAAACGGAGGA      80
81      GATTACGAATAAGCAATT      ATG GGC AAG CAA AAG CAG CAG CAG ACQ ATT TCT CGT TTC TTC GCT CCC      144
1      M G K Q K Q Q Q T I S R F A P      15
145     AAA CCC AAA TCC CCG ACT CAC GAA CCG AAT CCG GTA GCC GAA TCA TCA ACA CCG CCA CCG      204
16     K P K S P T H E P N P V A E S S T P P P      35
205     AAG ATA TCC GCC ACT GTA TCC TTC TCT CCT TCC AAG CGT AAG CTT CTC TCC GAC CAC CTC      264
36     K I S A T V S F S P S K R K L L S D H L      55
265     GCC GCC GCG TCA CCC AAA AAG CCT AAA CTT TCT CCT CAC ACT CAA AAC CCA GTA CCC GAT      324
56     A A A S P K K P K L S P H T Q N P V D      75
325     CCC AAT TTA CAC CAA AGA TTT CTC CAG AGA TTT CTG GAA CCC TCG CCG GAG GAA TAT GTT      384
76     P N L H Q R F L Q R F L E P S P E Y V      95
385     CCC GAA ACG TCA TCA TCG AGG AAA TAC ACA CCA TTT GAA CAG CAA GTG GTG GAG CTA AAG      444
96     P E T S S S R K Y T P L E Q V V E L K      115
445     AGC AAG TAC CCA GAT GTG GTT TTG ATG GTG GAA GTT GGT TAC AGG TAC AGA TTC TTC GGA      504
116     S K Y P D V V L M V E V G Y R Y R F F G      135
505     GAA GAC GCG GAG ATC GCA GCA CGC GTG TTG GGT ATT TAC GCT CAT ATG GAT CAC AAT TTC      564
136     E D A E I A A R V L G I Y A H M D H N F      155
565     ATG ACG GCG AGT GTG CCA ACA TTT CGA TTG AAT TTC CAT GTG AGA AGA CTG GTG AAT GCA      624
156     M T A S V P T F R L N F H V R R L V N A      175
625     GGA TAC AAG ATT GGT GTA GTG AAG CAG ACT GAA ACT GCA GCC ATT AAG TCC CAT GGT GCA      684
176     G Y K I G V V K Q T E T A A I K S H G A      195
665     AAC CGG ACC GCG CCT TTT TTC CGG GGA CTG TCG GCG TTG TAT ACC AAA GCC ACG CTT GAA      744
196     N R T G P F F R G L S A L Y T K A T L E      215
745     GCG GCT GAG GAT ATA AGT GGT GGT TGT GGT GAA GAA GGT TTT GGT TCA CAG AGT AAT      804
216     A A E D I S G G C G G E E G F G S Q S N      235
805     TTC TTG GTT TGT GTT GAT GAG AGA GTT AAG TCG GAG ACA TTA GGC TGT GGT ATT GAA      864
236     F L V C V V D E R V K S E T L G C G I E      255
865     ATG AGT TTT GAT GAT GAT GAT GAT GAT GAT GAT GAT GAT GAT GAT GAT GAT GAT GAT GAT      924
256     M S F D V R V G V G V G V G V G V G V G V G V G V G V G V G V G V G V G V G V      275

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Figure 4

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925 TAT GAA GAG TTC AAT GAT AAT TTC ATG AGA AGT GGA TTA GAG GCT GTG ATT TTG AGC TTG 984
 276 Y E E F N D N F M R S G L E A V I L S L 295
 985 TCA CCA GCT GAG CTG TTG CTT GGC CAG CCT CTT TCA CAA CAA ACT GAG AAG TTT TTG GTG 1044
 296 S P A E L L L G Q P L S Q Q T E K F L V 315
 1045 GCA CAT GCT GGA CCT ACC TCA AAC GTT CGA GTG GAA CGT GCC TCA CTG GAT TGT TTC AGC 1104
 316 A M A G P T S N V R V E R A S L D C F S 335
 1105 AAT GGT AAT GCA GTA GAT GAT GAT GAT GAT GAT GAT GAT GAT GAT GAT GAT GAT GAT 1164
 336 N G N A V D E V I S L C E K I S A G N L 355
 1165 GAA GAT GAT AAA GAA ATG AAG CTG GAG GCT GCT GAA AAA GGA ATG TCT TGC TTG ACA GTT 1224
 356 E D D K E M K L E A A E K G M S C L T V 375
 1225 CAT ACA ATT ATG AAC ATG CCA CAT CTG ACT GTT CAA GCC CTC GCC CTA ACG TTT TGC CAT 1284
 376 H T I M N M P H L T V Q A L A L T F C H 395
 1285 CTC AAA CAG TTT GGA TTT GAA AGG ATC CTT TAC CAA GGG GCC TCA TTT CGC TCT TTG TCA 1344
 396 L K Q F G F E R I L Y Q G A S F R S L S 415
 1345 AGT AAC ACA GAG ATG ACT CTC TCA GCC AAT ACT CTG CAA CAG TTG GAG GTT GTG AAA AAT 1404
 416 S N T E M T L S A N T L Q Q L E V V K N 435
 1405 AAT TCA GAT GGA TCG GAA TCT GGC TCC TTA TTC CAT AAT ATG AAT CAC ACA CTT ACA GTA 1464
 436 N S D G S E S G S L F H N M N H T L T V 455
 1465 TAT GCT TCC AGG CTT CTT AGA CAC TGG GTG ACT CAT CCT CTA TGC GAT AGA AAT TTG ATA 1524
 456 Y G S R L L R H W V T H P L C D R N L I 475
 1525 TCT GCT CGG CTT GAT GCT GTT TCT GAG ATT TCT GCT TGC ATG GGA TCT CAT AGT TCT TCC 1584
 476 S A R L D A V S E I S A C M G S H S S S 495
 1585 CAG CTC AGC AGT GAG TTG GTT GAA GAA GGT TCT TCT GAG AGA GCA ATT GTA TCA CCT GAG TTT 1644
 496 Q L S S E L V E E G S E R A I V S P E F 515
 1645 TAT CTC GTG CTC TCC TCA GTC TTG ACA GCT ATG TCT AGA TCA TCT GAT ATT CAA CGT GGA 1704
 516 Y L V L S S V L T A M S R S S D I Q R G 535
 1705 ATA ACA AGA ATC TTT CAT CGG ACT GCT AAA GCC ACA GAG TTC ATT GCA GTT ATG GAA GCT 1764
 536 I T R I F H R T A K A T E F I A V M E A 555
 1765 ATT TTA CTT GCG GGG AAG CAA ATT CAG CGG CTT GGC ATA AAG CAA GAC TCT GAA ATG AGG 1824
 556 I L L A G K Q I Q R L G I K Q D S E M R 575

Figure 4 (Continued)

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1825	AGT	ATG	CAA	TCT	GCA	ACT	GTG	CGA	TCT	ACT	CTT	TTG	AGA	AAA	TTG	ATT	TCT	GTT	ATT	TCA	1884
576	S	M	Q	S	A	T	V	R	S	T	L	L	R	K	L	I	S	V	I	S	595
1885	TCC	CCT	GTT	GTG	GTT	GAC	AAT	GCC	GGA	AAA	CTT	CTC	TCT	GCC	CTA	AAT	AAG	GAA	GCG	GCT	1944
596	S	P	V	V	V	D	N	A	G	K	L	L	S	A	L	N	K	E	A	A	615
1945	GTT	CGA	GGT	GAC	TTG	CTC	GAC	ATA	CTA	ATC	ACT	TCC	AGC	GAC	CAA	TTT	CCT	GAG	CTT	GCT	2004
616	V	R	G	D	L	L	D	I	L	I	T	S	S	D	Q	F	P	E	L	A	635
2005	GAA	GCT	CGC	CAA	GCA	GTT	TTA	GTC	ATC	AGG	GAA	AAG	CTG	GAT	TCC	TGG	ATA	GCT	TCA	TTT	2064
636	E	A	R	Q	A	V	L	V	I	R	E	K	L	D	S	I	A	S	F		655
2065	CGC	AAG	AAG	CTC	GCT	ATT	CGA	AAT	TTG	GAA	TTT	CTT	CAA	GTG	TGG	GGG	ATC	ACA	CAT	TTG	2124
656	R	K	K	L	A	I	R	N	L	E	F	L	Q	V	S	G	I	T	H	L	675
2125	ATA	GAG	CTG	CCC	GTT	GAT	TCC	AAG	GTC	CCT	ATG	AAT	TGG	GTG	AAA	GTA	AAT	AGC	ACC	AAG	2184
676	I	E	L	P	V	D	S	K	V	P	H	N	W	V	K	V	N	S	T	K	695
2185	AAG	ACT	ATT	CGA	TAT	CAT	CCC	CCA	GAA	ATA	GTA	GCT	GAC	TTG	GAT	GAG	CTA	GCT	CTA	GCA	2244
696	K	T	I	R	Y	H	P	P	E	I	V	A	G	L	D	E	L	A	L	A	715
2245	ACT	GAA	CAT	CTT	GCC	ATT	GTG	AAC	CGA	GCT	TGG	GAT	AGT	TTC	CTC	AAG	AGT	TTC	AGT		2304
716	T	E	H	L	A	I	V	N	R	A	S	W	D	S	F	L	K	S	F	S	735
2305	AGA	TAC	TAC	ACA	GAT	TTT	AAG	GCT	GCC	GTT	CAA	GCT	CTT	GCT	GCA	CTG	GAC	TGT	TTG	CAC	2364
736	R	Y	Y	T	D	F	K	A	A	V	Q	A	L	A	A	L	D	C	L	H	755
2365	TCC	CTT	TCA	ACT	CTA	TCT	ACA	AAC	AAG	AAC	TAT	GTC	CGT	CCC	GAG	TTT	GTG	GAT	GAC	TGT	2424
756	S	L	S	T	L	S	R	N	K	N	Y	V	R	P	E	F	V	D	C		775
2425	GAA	CCA	GTT	GAG	ATA	AAC	ATA	CAG	TCT	GGT	CGT	CAT	CCT	GTA	CTG	GAG	ACT	ATA	TTA	CAA	2484
776	E	P	V	E	I	N	I	Q	S	G	R	H	P	V	L	E	T	I	L	Q	795
2485	GAT	AAC	TTC	GTC	CCA	AAT	GAC	ACA	ATT	TTG	CAT	GCA	GAA	GGG	GAA	TAT	TGC	CAA	ATT	ATC	2544
796	D	N	F	V	P	N	D	T	I	L	H	A	E	G	E	Y	C	Q	I	I	815
2545	ACC	GGA	CCT	AAC	ATG	GGA	GGA	AAG	AGC	TGC	TAT	ATC	CGT	CAA	GTT	GCT	TTA	ATT	TCC	ATA	2604
816	T	G	P	N	M	G	G	K	S	C	Y	I	R	Q	V	A	L	I	S	I	835
2605	ATG	GCT	CAG	GTT	GGT	TGC	TTT	GTA	CCA	GCG	TCA	TTT	GCC	AAG	CTG	CAC	GTG	CTT	GAT	GGT	2664
836	M	A	Q	V	G	S	F	V	P	A	S	F	A	K	L	H	V	L	D	G	855
2665	GTT	TTC	ACT	CGG	ATG	GGT	GCT	TCA	GAC	AGT	ATC	CAG	CAT	GGC	AGA	AGT	ACC	TTT	CTA	GAA	2724
856	V	F	T	R	M	G	A	S	D	S	I	Q	H	G	R	S	T	F	L	E	875

Figure 4 (Continued)

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2725	GAA	TTA	AGT	GAA	GCG	TCA	CAC	ATA	ATC	AGA	ACC	TGT	TCT	TCT	TCT	CGT	TCG	CTT	GTT	ATA	TTA	2784
876	E	L	S	E	A	S	H	I	I	R	T	C	S	S	S	R	S	L	V	I	L	895
2785	GAT	GAG	CTT	GGA	AGA	GGC	ACT	AGC	ACA	CAC	GAC	GGT	GTA	GCC	ATT	GCC	TAT	GCA	ACA	TTA	2844	
896	D	E	L	G	R	G	T	S	T	H	D	G	V	A	I	A	Y	A	T	L	915	
2845	CAG	CAT	CTC	CTA	GCA	GAA	AAG	AGA	TGT	TTG	GTT	CTT	TTT	GTC	ACG	CAT	TAC	CCT	GAA	ATA	2904	
916	Q	H	L	L	A	E	K	R	C	L	V	L	F	V	T	H	Y	P	E	I	935	
2905	GCT	GAG	ATC	AGT	AAC	GGA	TTC	CCA	GGT	TCT	GTT	GGG	ACA	TAC	CAT	GTC	TCG	TAT	CTG	ACA	2964	
936	A	E	I	S	N	G	F	P	G	S	V	G	T	Y	H	V	S	Y	L	T	955	
2965	TTG	CAG	AAG	GAT	AAA	GGC	AGT	TAT	GAT	CAT	GAT	GAT	GTC	ACC	TAC	CTA	TAT	AAG	CTT	GTG	3024	
956	L	Q	K	D	K	G	S	Y	D	H	D	V	T	Y	L	Y	L	Y	K	L	V	975
3025	CGT	GGT	CTT	TGC	AGC	AGG	AGC	TTT	GGT	TTT	AAG	GTT	GCT	CAG	CTT	GCC	CAG	ATA	CCT	CCA	3084	
976	R	G	L	C	S	R	S	F	G	F	K	V	A	Q	L	A	Q	I	P	P	995	
3085	TCA	TGT	ATA	CGT	CGA	GCC	ATT	TCA	ATG	GCT	GCA	AAA	TTG	GAA	GCT	GAG	GTA	CGT	GCA	AGA	3144	
996	S	C	I	R	A	I	S	M	A	A	K	L	E	A	E	V	R	A	R		1015	
3145	GAG	AGA	AAT	ACA	CGC	ATG	GGA	GAA	CCA	GAA	GGA	CAT	GAA	CCG	AGA	GGC	GCA	GAA	GAA		3204	
1016	E	R	N	T	R	M	G	E	P	E	G	H	E	E	P	R	G	A	E	E	1035	
3205	TCT	ATT	TCG	GCT	CTA	GGT	GAC	TTG	TTT	GCA	GAC	CTG	AAA	TTT	GCT	CTC	TCT	GAA	GAG	GAC	3264	
1036	S	I	S	A	L	G	D	L	F	A	D	L	K	F	A	L	S	E	E	D	1055	
3265	CCT	TGG	AAA	GCA	TTC	GAG	TTT	TTA	AAG	CAT	GCT	TGG	AAG	ATT	GCT	GGC	AAA	ATC	AGA	CTA	3324	
1056	P	W	K	A	F	E	F	L	K	H	A	W	K	I	A	G	K	I	R	L	1075	
3325	AAA	CCA	ACT	TGT	TCA	TTT	TGA	TTTAACTTAA	CATTATAGCAACTGCAAGGCTCTTGA	TCATCTGTTAGTTGCG											3397	
1076	K	P	T	C	S	F	*														1082	
3398	TACTAACTT	ATG	TGT	ATT	AGT	ATA	ACA	AGA	AAA	GAG	AAT	TAG	AGAG	ATG	GAT	TCT	AAT	CCG				3458
1																					5	
3459	GTG	TTG	CAG	TAC	ATC	TTT	TCT	CCA	CCC	GCA	TAA	AAAAAAAAAAAAAAAAAAAA	AAAAAAAAAAAA									3522
6	V	L	Q	Y	I	F	S	P	P	A	*										16	

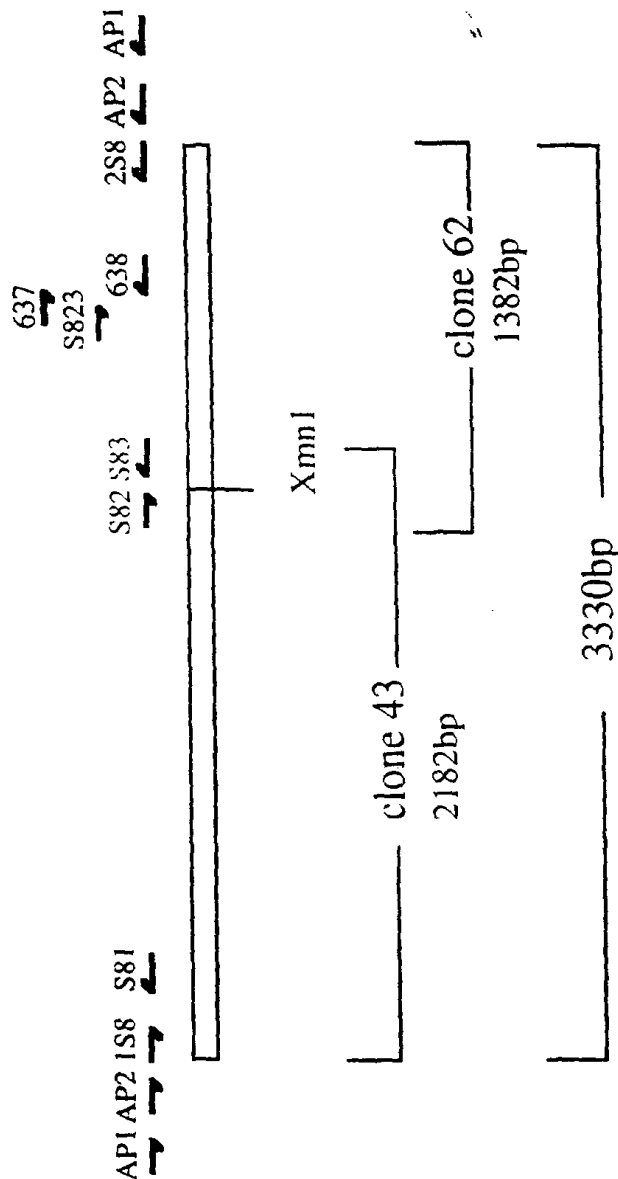
Figure 4 (Continued)

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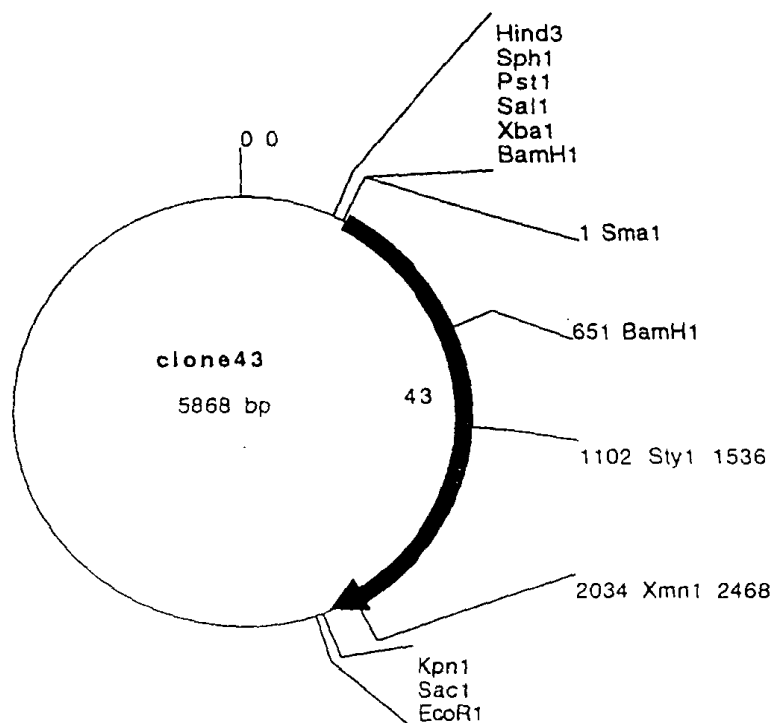
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Figure 6

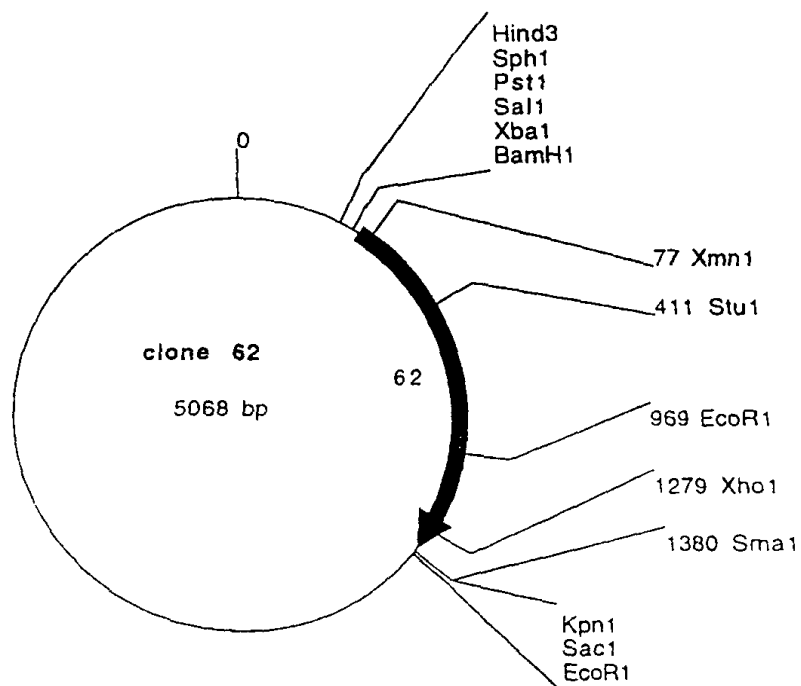


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**Figure 7**

Comments/References: 43= 5' side of S8 (AIMSH6) 2182 bp in pUC18/Sma1

422 Rec'd EST/PTO 10 APR 2000

**Figure 8**

Comments/References: 62= 3' side of S8 (AtMSH6) 1379bp in pUC18/Sma1

422 Rec'd [REDACTED] T/PTO 10 APR 2000

1	AAAAGTTGAGCCCTGAGGATATCGTTTCCGCCATTCTACGACGCAAGGGCAAAATTTTGGCGCCAATCTTTCCCCCC	80
81	TTTCGAATTCTCTCAGCTCAAAACATCGTTTCTCTCTCCTCTCTCTCTCACAATTCACAAAA	153
1		4
154	AGA TCG ATT TTG TCT TTC TTC CAA AAA CCC ACC GCG ACT ACG AAG GGT TTG GTT TCC	213
5	R S I L S F F Q K P T A A T T K G L V S	24
214	GGC GAT GCT GCT AGC GGC GGC GGC AGC GGA GGA CCA CGA TTT AAT GTG AAG GAA GGG	273
25	G D A A S G G G G G G S G G P , R F N V R E G	44
274	GAT GCT AAA GGC GAC GCT TCT GTA CGT TTT GCT GTT TCG AAA TCT GTC GAT GAG GTT AGA	333
45	D A K G D A S V R F A V S K S V D E V R	64
334	GGA ACG GAT ACT CCA CCG GAG AAG GTT CCG CGT GTC CTG CCG TCT GGA TTT AAG CCG	393
65	G T D T P P E K V P R P R V L P S G F K P	84
394	GCT GAA TCC GCC GST GAT GCT TCG TCC CTG TTC TCC AAT ATT ATG CAT AAG TTT GTA AAA	453
85	A E S A G D A S S L F S N I M H K F V K	104
454	GTC GAT GAT CGA GAT TGT TCT GGA GAG AGG AGC CGA GAA GAT GTT CCG CTG AAT GAT	513
105	V D D R D C S S G E R S R E D V V P L N D	124
514	TCA TCT CTA TGT ATG AAG GCT AAT GAT GTT ATT CCGT CAA TTT CGT TCC AAT AAT GGT AAA	573
125	S S L C M K A N D V I P Q F R S N N G K	144
574	ACT CAA GAA AGA AAC CAT GCT TTT AGT TTC AGT GGG AGA GCT GAA CTT AGA TCA GTA GAA	633
145	T Q E R N H A F S F S G R A E L R S V E	164
634	GAT ATA GGA GTA GAT GGC GAT GTT CCT GGT CCA GAA ACA CCA GGG ATG CCGT CCA CGT GCT	693
165	D I G V D G D V P G P E T P G M R P A	184
694	TCT CGC TTG AAG CGA GTT CTG GAG GAT GAA ATG ACT TTT AAG GAG GAT AAG GTT CCT GTA	753
185	S R L K R V L E D E M T F K E D K V P V	204
754	TTG GAC TCT AAC AAA AGG CTG AAA ATG CTC CAG GAT CCG GTT TGT GGA GAG AAG AAA GAA	813
205	L D S N K R L K M L Q D P V C G E K K E	224
814	GTA AAC GAA GGA ACC AAA TTT GAA TGG CTT GAG TCT TCT CGA ATC AGG GAT GCC AAT AGA	873
225	V N E G T K F E W L E S S R I R D A N R	244
874	AGA CGT CCT GAT GAT CCC CTT TAC GAT AGA AAG ACC TTA CAC ATA CCA CCT GAT GTT TTC	933
245	R R P D D P L Y D R K T L H I P P D V F	264

Figure 9

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934 AAG AAA ATG TCT GCA TCA CAA AAG CAA TAT TGG AGT GTT AAG AGT GAA TAT ATG GAC ATT 993
 265 K K M S A S Q K Q Y W S V K S E Y M D I 284
 996 GTG CTT TTC TTT AAA GTG GGG AAA TTT TAT GAG CTG TAT GAG CTA GAT GCG GAA TTA GGT 1053
 285 V L F F K V G K F Y E L Y E L D A E L G 304
 1054 CAC AAG GAG CTT GAC TGG AAG ATG ACC ATG AGT GGT GTG GGA AAA TGC AGA CAG GTT GGT 1113
 305 H K E L D W K M T M S G V G K C R Q V G 324
 1114 ATC TCT GAA AGT GGG ATA GAT GAG GCA GTG CAA AAG CTA TTA GCT CGT GGA TAT AAA GTT 1173
 325 I S E S G I D E A V Q K L L A R G Y K V 344
 1174 GGA CGA ATC GAG CAG CTA GAA ACA TCT GAC CAA GCA AAA GCC AGA GGT GCT AAT ACT ATA 1233
 345 G R I E Q L E T S D Q A K A R G A N T I 364
 1234 ATT CCA AGG AAG CTA GTT CAG GTA TTA ACT CCA TCA ACA GCA AGC GAG GGA AAC ATC GGG 1293
 365 I P R K L V Q V L T P S T A S E G N I G 384
 1294 CCT GAT GCC GTC CAT CTT GCT ATA AAA GAG ATC AAA ATG GAG CTA CAA AAG TGT TCA 1353
 385 P D A V H L A I K E I K M E L Q K C S 404
 1354 ACT GTG TAT GGA TTT GCT TTT GTT GAC TGT GCT GCC TTG AGG TTT TGG GTT GGG TCC ATC 1413
 405 T V Y G F A F V D C A A L R F W V G S I 424
 1414 AGC GAT GAT GCA TCA TGT GCT GCT CTT GGA GCG TTA TTG ATG CAG GTT TCT CCA AAG GAA 1473
 425 S D D A S C A A L G A L L M Q V S P K E 444
 1474 GTG TTA TAT GAC AGT AAA GGG CTA TCA AGA GAA GCA CAA AAG GCT CTA AGG AAA TAT ACG 1533
 445 V L Y D S K G L S R E A Q K A L R K Y T 464
 1534 TTG ACA GGG TCT ACG GCG GTA CAG TTG GCT CCA GTA CCA CAA GTA ATG GGG GAT ACA GAT 1593
 465 L T G S T A V Q L A P V P Q V M G D T D 484
 1594 GCT GCT GGA GTT AGA AAT ATA ATA GAA TCT AAC GGA TAC TTT AAA GGT TCT TCT GAA TCA 1653
 485 A A G V R N I I E S N G Y F K G S S E S 504
 1654 TGG AAC TGT GCT GTT GAT GGT CTA AAT GAA TGT GAT GTT GCC CTT AGT GCT CTT GGA GAG 1713
 505 W N C A V D G L N E C D V A L S A L G E 524
 1714 CTA ATT AAT CAT CTG TCT AGG CTA AAG CTA GAA GAT GTA CTT AAG CAT GGG GAT ATT TTT 1773
 525 L I N H L S R L K L E D V L K H G D I F 544
 1774 CCA TAC CAA GTT TAC AGG GGT TGT CTC AGA ATT GAT GGC CAG ACG ATG GTA AAT CTT GAG 1833
 545 P Y Q V Y R G C L R I D G Q T M V N L E 564

Figure 9 (Continued)

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1834 ATA TTT AAC AAT AGC TGT GAT GGT CCT TCA GGG ACC TTG TAC AAA TAT CTT GAT AAC 1893
 565 I F N N S C D G G P S G T L Y K Y L D N 584
 1894 TGT GTT AGT CCA ACT GGT AAG CGA CTC TTA AGG AAT TGG ATC TGC CAT CCA CTC AAA GAT 1953
 585 C V S P T G K R L L R N W I C H P L K D 604
 1954 GTA GAA AGC ATC AAT AAA CGG CTT GAT GTA GTT GAA GAA TTC ACG GCA AAC TCA GAA AGT 2013
 605 V E S I N K R L D V V E E F T A N S E S 624
 2014 ATG CAA ATC ACT GGC CAG TAT CTC CAC AAA CTT CCA GAC TTA GAA AGA CTG CTC GGA CGC 2073
 625 M Q I T G Q Y L H K L P D L E R L L G R 644
 2074 ATC AAG TCT AGC GTT CGA TCA TCA GCC TCT GTG TTG CTT GCT CTT CTG GGG AAA AAA GTG 2133
 645 I K S S V R S S A S V L P A L L G K K V 664
 2134 CTG AAA CAA CGA GTT AAA GCA TTT GGG CAA ATT GTG AAA GAG TTC AGA AGT GGA ATT GAT 2193
 665 L K Q R V K A F G Q I V K G F R S G I D 684
 2194 CTG TTG TTG GCT CTA CAG AAG GAA TCA AAT ATG ATG AGT TTG CTT TAT AAA CTC TGT AAA 2253
 685 L L A L Q K E S N M M S L L Y K L C K 704
 2254 CTT CCT ATA TTA GTA GGA AAA AGC GGG CTA GAG TTA TTT CTT TCT CAA TTC GAA GCA GCC 2313
 705 L P I L V G K S G L E L F L S Q F E A A 724
 2314 ATA GAT AGC GAC TTT CCA AAT TAT CAG AAC CAA GAT GTG ACA GAT GAA AAC GCT GAA ACT 2373
 725 I D S D F P N Y Q N Q D V T D E N A E T 744
 2374 CTC ACA ATA CTT ATC GAA CTT TTT ATC GAA AGA GCA ACT CAA TGG TCT GAG GTC ATT CAC 2433
 745 L T I L I E L F I E R A T Q W S E V I H 764
 2434 ACC ATA AGC TGC CTA GAT GTC CTG AGA TCT TTT GCA ATC GCA GCA AGT CTC TCT GCT GGA 2493
 765 T I S C L D V L R S F A I A A S L S A G 784
 2494 AGC ATG GCC AGG CCT GTT ATT TTT CCC GAA TCA GAA GCT ACA GAT CAG AAT CAG AAA ACA 2553
 785 S M A R P V I F P E S E A T D Q N Q K T 804
 2554 AAA GGG CCA ATA CTT AAA ATC CAA GGA CTA TGG CAT CCA TTT GCA GTT GCA GCC GAT GGT 2613
 805 K G P I L K I Q G L W H P F A V A A D G 824
 2614 CAA TTG CCT GTT CCG AAT GAT ATA CTC CTT GGC GAG GCT AGA AGC AGT GGC AGC ATT 2673
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Figure 9 (Continued)

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2794 885	GAA ATC TCC CTC GTG GAT ACT ATC TTC ACA AGG CTT GGC GCA TCT GAT AGA ATC ATG ACA	2853 904
2854 905	GGA GAG AGT ACC TTT TTG GTA GAA TGC ACT GAG ACA GCG TCA GTT CTT CAG AAT GCA ACT	2913 924
2914 925	CAG GAT TCA CTA GTA ATC CTT GAC GAA CTG GGC AGA GGA ACT AGT ACT TTC GAT GGA TAC	2973 944
2974 945	GCC ATT GCA TAC TCG GTT TTT CGT CAC CTG GTA GAG AAA GTT CAA TGT CGG ATG CTC TTT	3033 964
3034 965	GCA ACA CAT TAC CAC CCT CTC ACC AAG GAA TTC GCG TCT CAC CCA CGT GTC ACC TCG AAA	3093 984
3094 985	CAC ATG GCT TGC GCA TTC AAA TCA AGA TCT GAT TAT CAA CCA CGT GGT TGT GAT CAA GAC	3153 1004
3154 1005	CTA GTG TTC TTG TAC CGT TTA ACC GAG GGA GCT TGT CCT GAG AGC TAC GGA CTT CAA GTG	3213 1024
3214 1025	GCA CTC ATG GCT GGA ATA CCA AAC CAA GTG GTT GAA ACA GCA TCA GGT GCT CAA GCC	3273 1044
3274 1045	ATG AAG AGA TCA ATT GGG GGA AAC TTC AAG TCA AGT CAG CTA AGA TCT GAG TTC TCA AGT	3333 1064
3334 1065	CTG CAT GAA GAC TGG CTC AAG TCA TTG GTG GGT ATT TCT CGA GTC GCC CAC AAC AAT GCC	3393 1084
3394 1085	CCC ATT GGC GAA GAT GAC TAC GAC ACT TTG TTT TGC TTA TGG CAT GAG ATC AAA TCC TCT	3453 1104
3454 1105	TAC TGT GTT CCC AAA TAA ATG GCT ATG ACA TAA CACTATCTGAAGCTCGTTAAGTCTTTTGCCTCTCT	3521 5
3522 1	G ATG TTT ATT CCT CTT AAA AAA TGC TTA TAT ATC AAA AAA TTG TTT CCT CGA TTA AAA	3579 19
3580 20	AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA AAA	3606 28

Figure 9 (Continued)

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[illegible]

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Figure 11

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Figure 11 (Continued)

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Figure 11 (Continued)

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Figure 11 (Continued)

422 Rec'd PCT/STO 1 0 APR 2000.

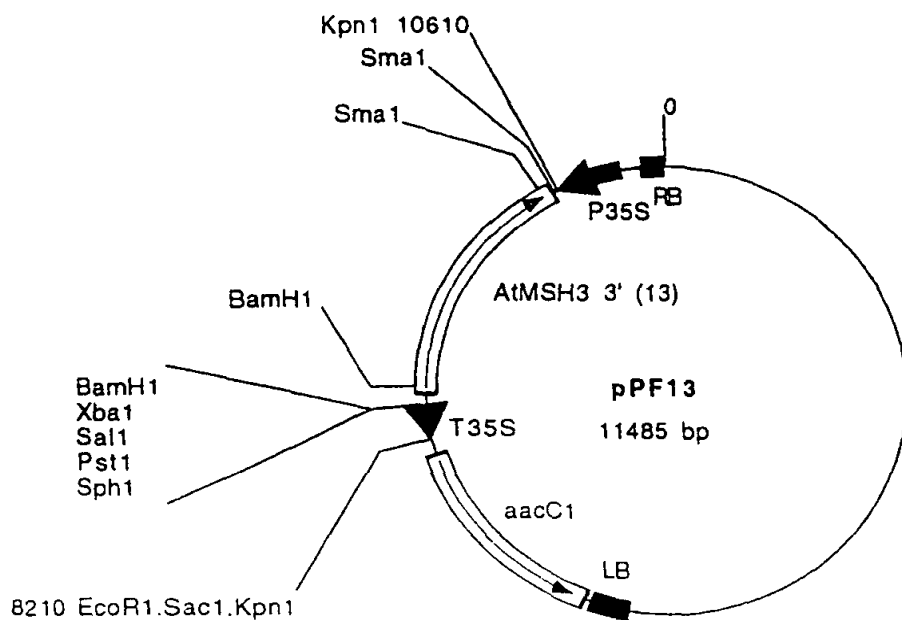


Figure 12

Comments/References: AtMSH3 3' side antisense : AtMSH3 3' (13 = 2104bp) from pUC18/13 Sal1/Sst1/T4 into pCW164 BamH1/T4 in Agrobacterium LBA44O4

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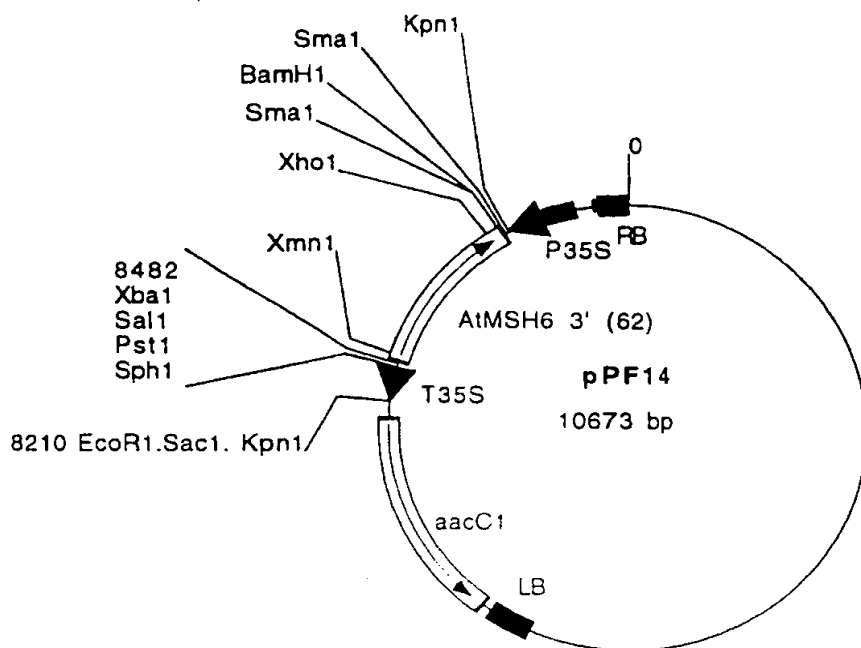


Figure 13

Comments/References: AtMSH6 (S8) 3' side antisens : 62 SalI/SstI/T4 (1379bp)
into pCW164 BamHI/T4

422 Rec'd PST/PTO 1 0 APR 2000

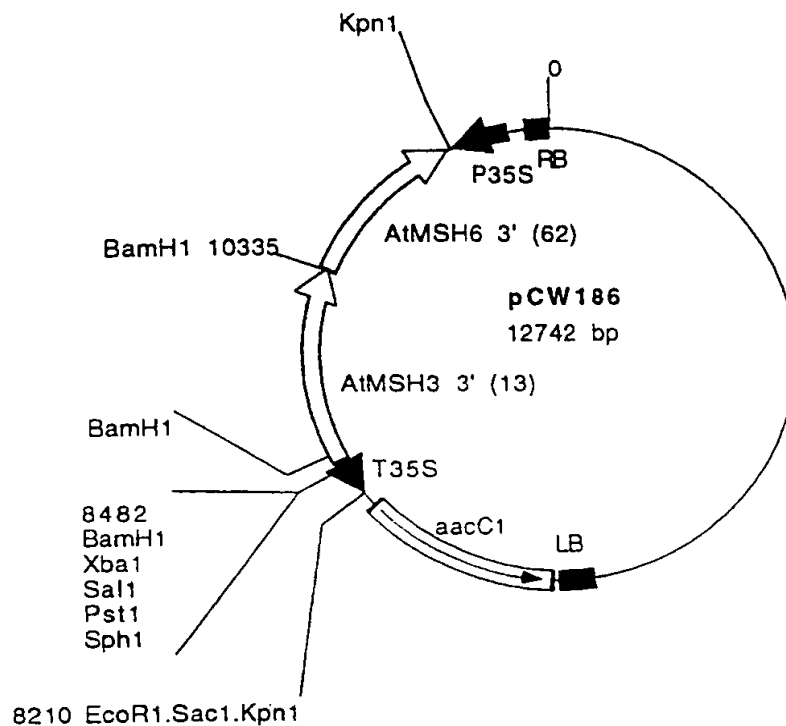


Figure 14

Comments/References: AtMSH6 3'/AtMSH3 3' antisense : AtMSH6 (S8) 3' side (62=1379bp)
SalI/SstI/T4 into pPF13 (pCW164 AtMSH3 (S5) 3' side (13=2104) antisense)/SmaI. in
LBA4404

422 Rec'

T/PTO 10 APR 2000

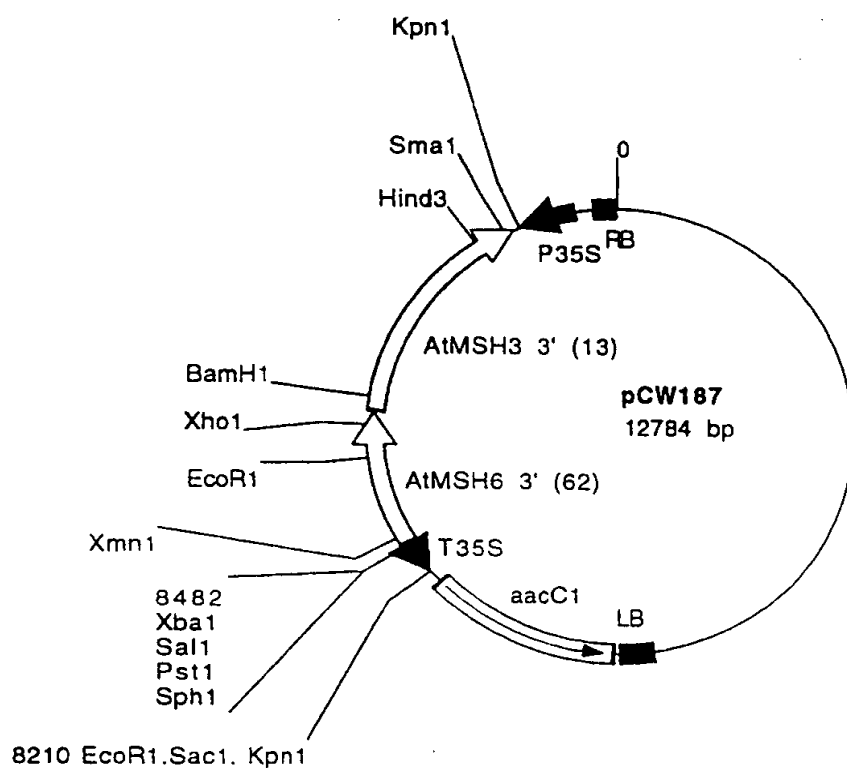


Figure 15

Comments/References: AtMSH3 3'/AtMSH6 3' antisens (D) : AtMSH3 (S5) 3' side (13=2104bp) Sal1/Sst1/T4 into pPF14 (AtMSH6 (S8) 3'side (62=1379bp) antisense into pCW164)/Sma1. in LBA4404

422 Rec'd PO TO 1 0 APR 2000

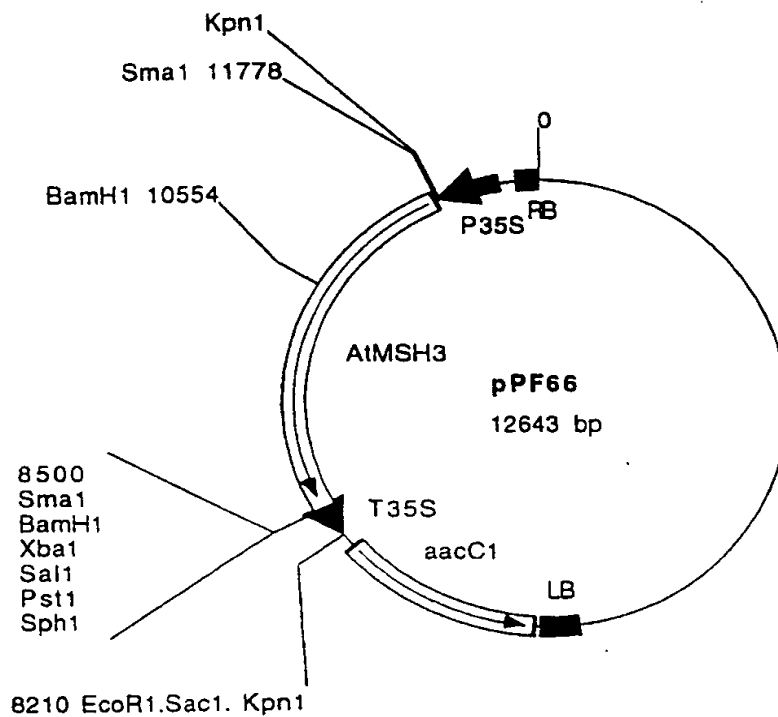


Figure 16

Comments/References: AtMSH3 (S8) complete, sense orientation : pPF26 (3342bp)
Sma1 into pCW164 Sma1

422 Rec'd PCT/PTO 1 0 APR 2000

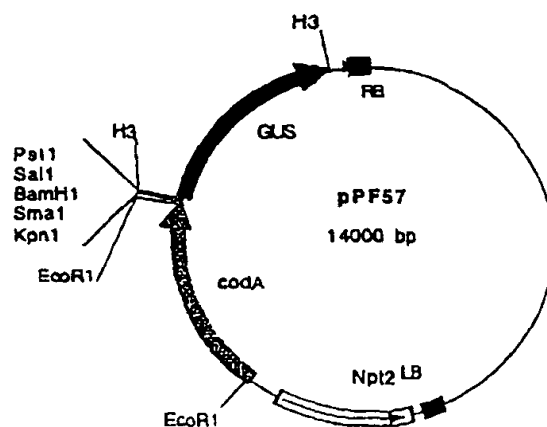
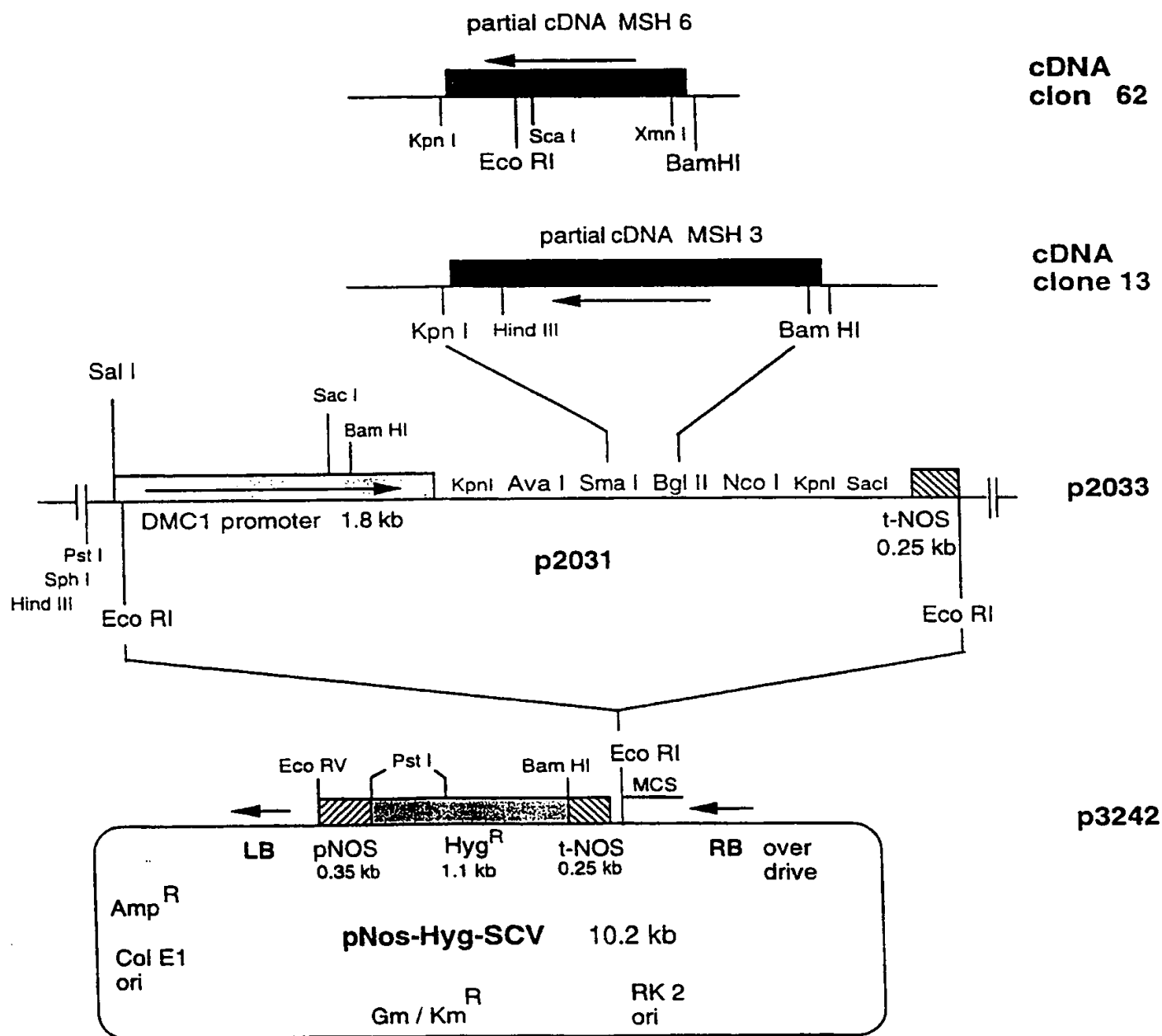


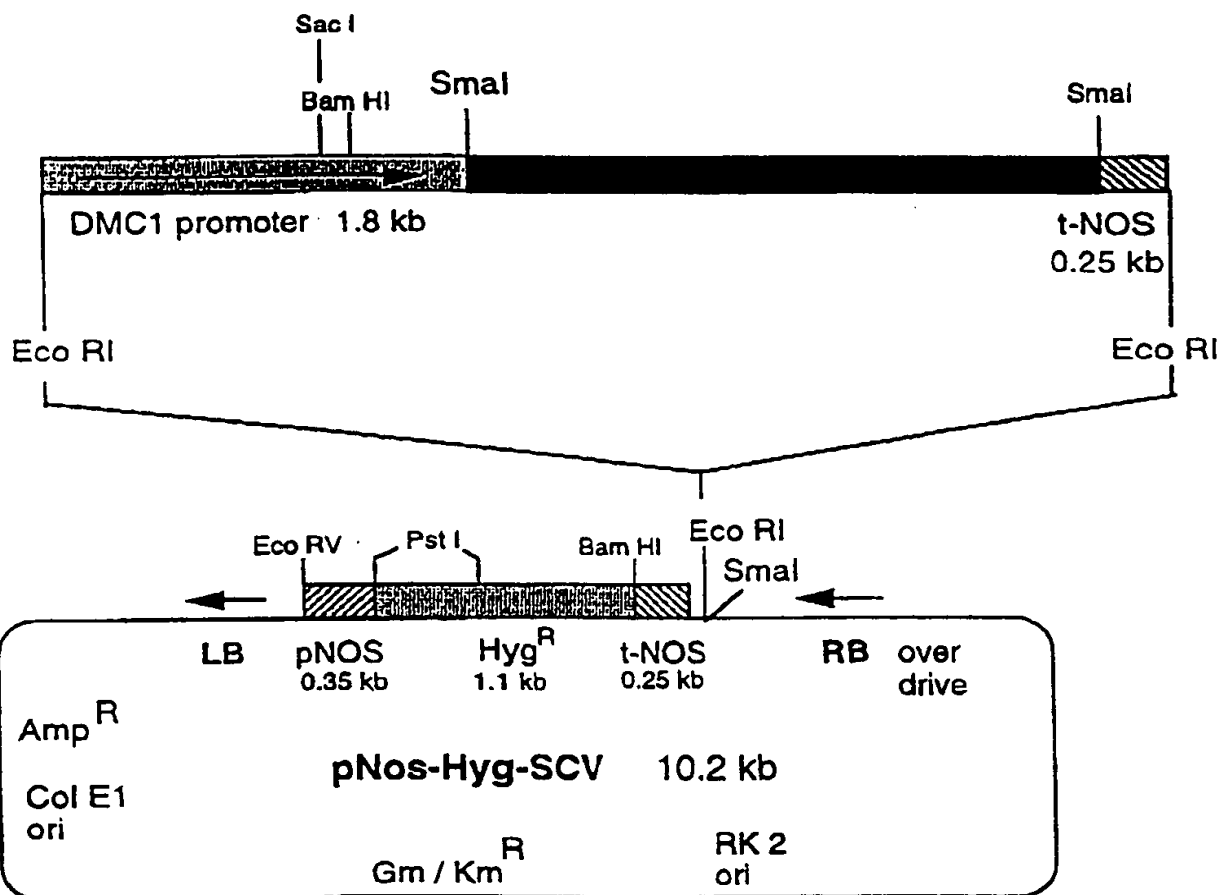
Figure 17

Comments/References: pPZP111 with codA EcoR1 cassette in EcoR1 site and Hind3 GUS cassette in Hind3 site. KanR. All genes under Promoter/terminator 35S

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Figure 18

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Figure 19**p3243**

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SEQUENCE LISTING

<110> Rhone-Poulenc Agro; Betzner, Andreas Stefan; Doutriaux, Marie-Pascale; Freyssinet, Georges; Perez, Pascual.

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<212> DNA

<213> Arabidopsis thaliana ecotype Columbia

<223> Clone 52

<400>

12

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gccactgtat ctttctctcc ttccaagcgt aagcttctct ccgaccacct cgccgccgcg      180
tcacccaaaa agcctaaact ttctcctcac actcaaaacc cagtaccgga tcccaattta      240
caccaaagat ttctccagag atttctggaa ccttcgccgg aggaatatgt tcccgaaacg      300
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ccagatgtgg ttttgatggg ggaagttggg tacaggtaca gattcttcgg agaagacgcg      420
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<210>

13

<211>

34

<212>

DNA

<213>

Artificial sequence

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<223>

MSH3 specific primer 2S5 for PCR using cDNA of *Arabidopsis thaliana* ecotype Columbia

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34

<210> 14

<211> 27

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<213> Artificial sequence

<220>

<223> MSH3 specific primer S52 for PCR using cDNA of Arabidopsis thaliana ecotype Columbia

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27

<210> 15

<211> 2110

<212> DNA

<213> Arabidopsis thaliana ecotype Columbia

<223> Clone 13

<400> 15

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tctctcagcc aatactctgc aacagtggga gggtgtgaaa aataattcag atggatcgga 180

atctggctcc ttattccata atatgaatca cacacttaca gtatatgggt ccaggcttct 240

tagacactgg gtgactcatc ctctatgcga tagaaatttg atatctgctc ggcttgatgc 300

tgtttctgag atttctgctt gcatgggatc tcatagttct tcccagctca gcagtgagtt 360

ggttgaagaa ggttctgaga gagcaattgt atcacctgag ttttatctcg tgctctctc 420

agtcttgaca gctatgtcta gatcatctga tattcaacgt ggaataacaa gaatctttca 480

tcggactgct aaagccacag agttcattgc agttatggaa gctattttac ttgcggggaa 540

gcaaattcag cggcttggca taaagcaaga ctctgaaatg aggagtatgc aatctgcaac 600

tgtgcatctt actcttttga gaaaattgat ttctgttatt tcatccctg ttgtgggtga 660

caatgccgga aaacttctct ctgccctaaa taaggaagcg gctgttcgag gtgacttgct 720

cgacatacta atcacttcca gcgaccaatt tcctgagctt gctgaagctc gccaaagcagt 780

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tgacacaatt ttgcatgcag aaggggaata ttgccaaatt atcaccggac ctaacatggg     1320
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<211> 29

<212> DNA

<213> Artificial sequence

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<223> MSH3 specific primer S51 for PCR using cDNA of Arabidopsis thaliana ecotype Columbia

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<210> 17
 <211> 30
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 <213> Artificial sequence

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 1 5 10 15

ccc aaa tcc ccg act cac gaa ccg aat ccg gta gcc gaa tca tca aca 195
 Pro Lys Ser Pro Thr His Glu Pro Asn Pro Val Ala Glu Ser Ser Thr
 20 25 30

ccg cca ccg aag ata tcc gcc act gta tcc ttc tct cct tcc aag cgt 243
 Pro Pro Pro Lys Ile Ser Ala Thr Val Ser Phe Ser Pro Ser Lys Arg
 35 40 45

aag ctt ctc tcc gac cac ctc gcc gcc gcg tca ccc aaa aag cct aaa 291
 Lys Leu Leu Ser Asp His Leu Ala Ala Ala Ser Pro Lys Lys Pro Lys
 50 55 60

ctt tct cct cac act caa aac cca gta ccc gat ccc aat tta cac caa 339
 Leu Ser Pro His Thr Gln Asn Pro Val Pro Asp Pro Asn Leu His Gln
 65 70 75 80

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Arg Phe Leu Gln Arg Phe Leu Glu Pro Ser Pro Glu Glu Tyr Val Pro	
85 90 95	
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Glu Thr Ser Ser Ser Arg Lys Tyr Thr Pro Leu Glu Gln Gln Val Val	
100 105 110	
gag cta aag agc aag tac cca gat gtg gtt ttg atg gtg gaa gtt ggt	483
Glu Leu Lys Ser Lys Tyr Pro Asp Val Val Leu Met Val Glu Val Gly	
115 120 125	
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Tyr Arg Tyr Arg Phe Phe Gly Glu Asp Ala Glu Ile Ala Ala Arg Val	
130 135 140	
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Leu Gly Ile Tyr Ala His Met Asp His Asn Phe Met Thr Ala Ser Val	
145 150 155 160	
cca aca ttt cga ttg aat ttc cat gtg aga aga ctg gtg aat gca gga	627
Pro Thr Phe Arg Leu Asn Phe His Val Arg Arg Leu Val Asn Ala Gly	
165 170 175	
tac aag att ggt gta gtg aag cag act gaa act gca gcc att aag tcc	675
Tyr Lys Ile Gly Val Val Lys Gln Thr Glu Thr Ala Ala Ile Lys Ser	
180 185 190	
cat ggt gca aac cgg acc ggc cct ttt ttc cgg gga ctg tcg gcg ttg	723
His Gly Ala Asn Arg Thr Gly Pro Phe Phe Arg Gly Leu Ser Ala Leu	
195 200 205	
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Tyr Thr Lys Ala Thr Leu Glu Ala Ala Glu Asp Ile Ser Gly Gly Cys	
210 215 220	
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225 230 235 240	
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Val Asp Glu Arg Val Lys Ser Glu Thr Leu Gly Cys Gly Ile Glu Met	
245 250 255	
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Ser Phe Asp Val Arg Val Gly Val Val Gly Val Glu Ile Ser Thr Gly	
260 265 270	
gaa gtt gtt tat gaa gag ttc aat gat aat ttc atg aga agt gga tta	963
Glu Val Val Tyr Glu Glu Phe Asn Asp Asn Phe Met Arg Ser Gly Leu	
275 280 285	

10

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Pro Leu Ser Gln Gln Thr Glu Lys Phe Leu Val Ala Met Ala Gly Pro	
305 310 315 320	
acc tca aac gtt cga gtg gaa cgt gcc tca ctg gat tgt ttc agc aat	1107
Thr Ser Asn Val Arg Val Glu Arg Ala Ser Leu Asp Cys Phe Ser Asn	
325 330 335	
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Gly Asn Ala Val Asp Glu Val Ile Ser Leu Cys Glu Lys Ile Ser Ala	
340 345 350	
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Gly Asn Leu Glu Asp Asp Lys Glu Met Lys Leu Glu Ala Ala Glu Lys	
355 360 365	
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Gly Met Ser Cys Leu Thr Val His Thr Ile Met Asn Met Pro His Leu	
370 375 380	
act gtt caa gcc ctg gcc cta acg ttt tgc cat ctg aaa cag ttt gga	1299
Thr Val Gln Ala Leu Ala Leu Thr Phe Cys His Leu Lys Gln Phe Gly	
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405 410 415	
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Asn Thr Glu Met Thr Leu Ser Ala Asn Thr Leu Gln Gln Leu Glu Val	
420 425 430	
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Val Lys Asn Asn Ser Asp Gly Ser Glu Ser Gly Ser Leu Phe His Asn	
435 440 445	
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Met Asn His Thr Leu Thr Val Tyr Gly Ser Arg Leu Leu Arg His Trp	
450 455 460	
gtg act cat cct cta tgc gat aga aat ttg ata tct gct cgg ctt gat	1539
Val Thr His Pro Leu Cys Asp Arg Asn Leu Ile Ser Ala Arg Leu Asp	
465 470 475 480	
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Ala Val Ser Glu Ile Ser Ala Cys Met Gly Ser His Ser Ser Ser Gln	
485 490 495	

ctc agc agt gag ttg gtt gaa gaa ggt tct gag aga gca att gta tca	1635
Leu Ser Ser Glu Leu Val Glu Glu Gly Ser Glu Arg Ala Ile Val Ser	
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cct gag ttt tat ctc gtg ctc tcc tca gtc ttg aca gct atg tct aga	1683
Pro Glu Phe Tyr Leu Val Leu Ser Ser Val Leu Thr Ala Met Ser Arg	
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Ser Ser Asp Ile Gln Arg Gly Ile Thr Arg Ile Phe His Arg Thr Ala	
530 535 540	
aaa gcc aca gag ttc att gca gtt atg gaa gct att tta ctt gcg ggg	1779
Lys Ala Thr Glu Phe Ile Ala Val Met Glu Ala Ile Leu Leu Ala Gly	
545 550 555 560	
aag caa att cag cgg ctt ggc ata aag caa gac tct gaa atg agg agt	1827
Lys Gln Ile Gln Arg Leu Gly Ile Lys Gln Asp Ser Glu Met Arg Ser	
565 570 575	
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Met Gln Ser Ala Thr Val Arg Ser Thr Leu Leu Arg Lys Leu Ile Ser	
580 585 590	
gtt att tca tcc cct gtt gtg gtt gac aat gcc gga aaa ctt ctc tct	1923
Val Ile Ser Ser Pro Val Val Val Asp Asn Ala Gly Lys Leu Leu Ser	
595 600 605	
gcc cta aat aag gaa gcg gct gtt cga ggt gac ttg ctc gac ata cta	1971
Ala Leu Asn Lys Glu Ala Ala Val Arg Gly Asp Leu Leu Asp Ile Leu	
610 615 620	
atc act tcc agc gac caa ttt cct gag ctt gct gaa gct cgc caa gca	2019
Ile Thr Ser Ser Asp Gln Phe Pro Glu Leu Ala Glu Ala Arg Gln Ala	
625 630 635 640	
gtt tta gtc atc agg gaa aag ctg gat tcc tcg ata gct tca ttt cgc	2067
Val Leu Val Ile Arg Glu Lys Leu Asp Ser Ser Ile Ala Ser Phe Arg	
645 650 655	
aag aag ctc gct att cga aat ttg gaa ttt ctt caa gtg tcg ggg atc	2115
Lys Lys Leu Ala Ile Arg Asn Leu Glu Phe Leu Gln Val Ser Gly Ile	
660 665 670	
aca cat ttg ata gag ctg ccc gtt gat tcc aag gtc cct atg aat tgg	2163
Thr His Leu Ile Glu Leu Pro Val Asp Ser Lys Val Pro His Asn Trp	
675 680 685	
gtg aaa gta aat agc acc aag aag act att cga tat cat ccc cca gaa	2211
Val Lys Val Asn Ser Thr Lys Lys Thr Ile Arg Tyr His Pro Pro Glu	
690 695 700	

ata gta gct ggc ttg gat gag cta gct cta gca act gaa cat ctt gcc	2259
Ile Val Ala Gly Leu Asp Glu Leu Ala Leu Ala Thr Glu His Leu Ala	
705 710 715 720	
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Ile Val Asn Arg Ala Ser Trp Asp Ser Phe Leu Lys Ser Phe Ser Arg	
725 730 735	
tac tac aca gat ttt aag gct gcc gtt caa gct ctt gct gca ctg gac	2355
Tyr Tyr Thr Asp Phe Lys Ala Ala Val Gln Ala Leu Ala Ala Leu Asp	
740 745 750	
tgt ttg cac tcc ctt tca act cta tct aga aac aag aac tat gtc cgt	2403
Cys Leu His Ser Leu Ser Thr Leu Ser Arg Asn Lys Asn Tyr Val Arg	
755 760 765	
ccc gag ttt gtg gat gac tgt gaa cca gtt gag ata aac ata cag tct	2451
Pro Glu Phe Val Asp Asp Cys Glu Pro Val Glu Ile Asn Ile Gln Ser	
770 775 780	
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Gly Arg His Pro Val Leu Glu Thr Ile Leu Gln Asp Asn Phe Val Pro	
785 790 795 800	
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Gly Pro Asn Met Gly Gly Lys Ser Cys Tyr Ile Arg Gln Val Ala Leu	
820 825 830	
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Ile Ser Ile Met Ala Gln Val Gly Ser Phe Val Pro Ala Ser Phe Ala	
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Lys Leu His Val Leu Asp Gly Val Phe Thr Arg Met Gly Ala Ser Asp	
850 855 860	
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Ser Ile Gln His Gly Arg Ser Thr Phe Leu Glu Glu Leu Ser Glu Ala	
865 870 875 880	
tca cac ata atc aga acc tgt tct tct cgt tcg ctt gtt ata tta gat	2787
Ser His Ile Ile Arg Thr Cys Ser Ser Arg Ser Leu Val Ile Leu Asp	
885 890 895	
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Glu Leu Gly Arg Gly Thr Ser Thr His Asp Gly Val Ala Ile Ala Tyr	
900 905 910	

13

gca aca tta cag cat ctc cta gca gaa aag aga tgt ttg gtt ctt ttt	2883
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gtc acg cat tac cct gaa ata gct gag atc agt aac gga ttc cca ggt	2931
Val Thr His Tyr Pro Glu Ile Ala Glu Ile Ser Asn Gly Phe Pro Gly	
930 935 940	
tct gtt ggg aca tac cat gtc tcg tat ctg aca ttg cag aag gat aaa	2979
Ser Val Gly Thr Tyr His Val Ser Tyr Leu Thr Leu Gln Lys Asp Lys	
945 950 955 960	
ggc agt tat gat cat gat gat gtg acc tac cta tat aag ctt gtg cgt	3027
Gly Ser Tyr Asp His Asp Asp Val Thr Tyr Leu Tyr Lys Leu Val Arg	
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Gly Leu Cys Ser Arg Ser Phe Gly Phe Lys Val Ala Gln Leu Ala Gln	
980 985 990	
ata cct cca tca tgt ata cgt cga gcc att tca atg gct gca aaa ttg	3123
Ile Pro Pro Ser Cys Ile Arg Arg Ala Ile Ser Met Ala Ala Lys Leu	
995 1000 1005	
gaa gct gag gta cgt gca aga gag aga aat aca cgc atg gga gaa cca	3171
Glu Ala Glu Val Arg Ala Arg Glu Arg Asn Thr Arg Met Gly Glu Pro	
1010 1015 1020	
gaa gga cat gaa gaa ccg aga ggc gca gaa gaa tct att tcg gct cta	3219
Glu Gly His Glu Glu Pro Arg Gly Ala Glu Glu Ser Ile Ser Ala Leu	
1025 1030 1035 1040	
ggg gac ttg ttt gca gac ctg aaa ttt gct ctc tct gaa gag gac cct	3267
Gly Asp Leu Phe Ala Asp Leu Lys Phe Ala Leu Ser Glu Glu Asp Pro	
1045 1050 1055	
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Trp Lys Ala Phe Glu Phe Leu Lys His Ala Trp Lys Ile Ala Gly Lys	
1060 1065 1070	
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Ile Arg Leu Lys Pro Thr Cys Ser Phe	
1075 1080	
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<213> *Arabidopsis thaliana* ecotype Columbia
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<400> 19

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Pro	Pro	Pro	Lys	Ile	Ser	Ala	Thr	Val	Ser	Phe	Ser	Pro	Ser	Lys	Arg	35	40	45	
Lys	Leu	Leu	Ser	Asp	His	Leu	Ala	Ala	Ala	Ser	Pro	Lys	Lys	Pro	Lys	50	55	60	
Leu	Ser	Pro	His	Thr	Gln	Asn	Pro	Val	Pro	Asp	Pro	Asn	Leu	His	Gln	65	70	75	80
Arg	Phe	Leu	Gln	Arg	Phe	Leu	Glu	Pro	Ser	Pro	Glu	Glu	Tyr	Val	Pro	85	90	95	
Glu	Thr	Ser	Ser	Ser	Arg	Lys	Tyr	Thr	Pro	Leu	Glu	Gln	Gln	Val	Val	100	105	110	
Glu	Leu	Lys	Ser	Lys	Tyr	Pro	Asp	Val	Val	Leu	Met	Val	Glu	Val	Gly	115	120	125	
Tyr	Arg	Tyr	Arg	Phe	Phe	Gly	Glu	Asp	Ala	Glu	Ile	Ala	Ala	Arg	Val	130	135	140	
Leu	Gly	Ile	Tyr	Ala	His	Met	Asp	His	Asn	Phe	Met	Thr	Ala	Ser	Val	145	150	155	160
Pro	Thr	Phe	Arg	Leu	Asn	Phe	His	Val	Arg	Arg	Leu	Val	Asn	Ala	Gly	165	170	175	
Tyr	Lys	Ile	Gly	Val	Val	Lys	Gln	Thr	Glu	Thr	Ala	Ala	Ile	Lys	Ser	180	185	190	
His	Gly	Ala	Asn	Arg	Thr	Gly	Pro	Phe	Phe	Arg	Gly	Leu	Ser	Ala	Leu	195	200	205	
Tyr	Thr	Lys	Ala	Thr	Leu	Glu	Ala	Ala	Glu	Asp	Ile	Ser	Gly	Gly	Cys	210	215	220	
Gly	Gly	Glu	Glu	Gly	Phe	Gly	Ser	Gln	Ser	Asn	Phe	Leu	Val	Cys	Val	225	230	235	240
Val	Asp	Glu	Arg	Val	Lys	Ser	Glu	Thr	Leu	Gly	Cys	Gly	Ile	Glu	Met	245	250	255	

Ser Phe Asp Val Arg Val Gly Val Val Gly Val Glu Ile Ser Thr Gly
 260 265 270
 Glu Val Val Tyr Glu Glu Phe Asn Asp Asn Phe Met Arg Ser Gly Leu
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 Glu Ala Val Ile Leu Ser Leu Ser Pro Ala Glu Leu Leu Gly Gln
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 Pro Leu Ser Gln Gln Thr Glu Lys Phe Leu Val Ala Met Ala Gly Pro
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 Thr Ser Asn Val Arg Val Glu Arg Ala Ser Leu Asp Cys Phe Ser Asn
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 Gly Asn Ala Val Asp Glu Val Ile Ser Leu Cys Glu Lys Ile Ser Ala
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 Gly Met Ser Cys Leu Thr Val His Thr Ile Met Asn Met Pro His Leu
 370 375 380
 Thr Val Gln Ala Leu Ala Leu Thr Phe Cys His Leu Lys Gln Phe Gly
 385 390 395 400
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 405 410 415
 Asn Thr Glu Met Thr Leu Ser Ala Asn Thr Leu Gln Gln Leu Glu Val
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 Val Lys Asn Asn Ser Asp Gly Ser Glu Ser Gly Ser Leu Phe His Asn
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 Met Asn His Thr Leu Thr Val Tyr Gly Ser Arg Leu Leu Arg His Trp
 450 455 460
 Val Thr His Pro Leu Cys Asp Arg Asn Leu Ile Ser Ala Arg Leu Asp
 465 470 475 480
 Ala Val Ser Glu Ile Ser Ala Cys Met Gly Ser His Ser Ser Ser Gln
 485 490 495
 Leu Ser Ser Glu Leu Val Glu Glu Gly Ser Glu Arg Ala Ile Val Ser
 500 505 510
 Pro Glu Phe Tyr Leu Val Leu Ser Ser Val Leu Thr Ala Met Ser Arg
 515 520 525
 Ser Ser Asp Ile Gln Arg Gly Ile Thr Arg Ile Phe His Arg Thr Ala
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Lys	Ala	Thr	Glu	Phe	Ile	Ala	Val	Met	Glu	Ala	Ile	Leu	Leu	Ala	Gly	545	550	555	560
Lys	Gln	Ile	Gln	Arg	Leu	Gly	Ile	Lys	Gln	Asp	Ser	Glu	Met	Arg	Ser	565	570	575	
Met	Gln	Ser	Ala	Thr	Val	Arg	Ser	Thr	Leu	Leu	Arg	Lys	Leu	Ile	Ser	580	585	590	
Val	Ile	Ser	Ser	Pro	Val	Val	Val	Asp	Asn	Ala	Gly	Lys	Leu	Leu	Ser	595	600	605	
Ala	Leu	Asn	Lys	Glu	Ala	Ala	Val	Arg	Gly	Asp	Leu	Leu	Asp	Ile	Leu	610	615	620	
Ile	Thr	Ser	Ser	Asp	Gln	Phe	Pro	Glu	Leu	Ala	Glu	Ala	Arg	Gln	Ala	625	630	635	640
Val	Leu	Val	Ile	Arg	Glu	Lys	Leu	Asp	Ser	Ser	Ile	Ala	Ser	Phe	Arg	645	650	655	
Lys	Lys	Leu	Ala	Ile	Arg	Asn	Leu	Glu	Phe	Leu	Gln	Val	Ser	Gly	Ile	660	665	670	
Thr	His	Leu	Ile	Glu	Leu	Pro	Val	Asp	Ser	Lys	Val	Pro	His	Asn	Trp	675	680	685	
Val	Lys	Val	Asn	Ser	Thr	Lys	Lys	Thr	Ile	Arg	Tyr	His	Pro	Pro	Glu	690	695	700	
Ile	Val	Ala	Gly	Leu	Asp	Glu	Leu	Ala	Leu	Ala	Thr	Glu	His	Leu	Ala	705	710	715	720
Ile	Val	Asn	Arg	Ala	Ser	Trp	Asp	Ser	Phe	Leu	Lys	Ser	Phe	Ser	Arg	725	730	735	
Tyr	Tyr	Thr	Asp	Phe	Lys	Ala	Ala	Val	Gln	Ala	Leu	Ala	Ala	Leu	Asp	740	745	750	
Cys	Leu	His	Ser	Leu	Ser	Thr	Leu	Ser	Arg	Asn	Lys	Asn	Tyr	Val	Arg	755	760	765	
Pro	Glu	Phe	Val	Asp	Asp	Cys	Glu	Pro	Val	Glu	Ile	Asn	Ile	Gln	Ser	770	775	780	
Gly	Arg	His	Pro	Val	Leu	Glu	Thr	Ile	Leu	Gln	Asp	Asn	Phe	Val	Pro	785	790	795	800
Asn	Asp	Thr	Ile	Leu	His	Ala	Glu	Gly	Glu	Tyr	Cys	Gln	Ile	Ile	Thr	805	810	815	
Gly	Pro	Asn	Met	Gly	Gly	Lys	Ser	Cys	Tyr	Ile	Arg	Gln	Val	Ala	Leu	820	825	830	

17

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 850 855 860
 Ser Ile Gln His Gly Arg Ser Thr Phe Leu Glu Glu Leu Ser Glu Ala
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 Ser His Ile Ile Arg Thr Cys Ser Ser Arg Ser Leu Val Ile Leu Asp
 885 890 895
 Glu Leu Gly Arg Gly Thr Ser Thr His Asp Gly Val Ala Ile Ala Tyr
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 Val Thr His Tyr Pro Glu Ile Ala Glu Ile Ser Asn Gly Phe Pro Gly
 930 935 940
 Ser Val Gly Thr Tyr His Val Ser Tyr Leu Thr Leu Gln Lys Asp Lys
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 965 970 975
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<210> 21
<211> 28
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<210> 24
<211> 33
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<213> Artificial sequence

<220>

<223> MSH6 specific primer 1S8 for PCR using cDNA of Arabidopsis thaliana ecotype Columbia

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<210> 25

<211> 27

<212> DNA

<213> Artificial sequence

<220>

<223> MSH6 specific primer S83 for PCR using cDNA of Arabidopsis thaliana ecotype Columbia

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cgctatctat ggctgcttcg aattgag 27

<210> 26

<211> 1385

<212> DNA

<213> Arabidopsis thaliana ecotype Columbia

<223> Clone 43

<400> 26

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tttaatgtga aggaagggga tgctaaaggc gacgcttctg tacgttttgc tgtttcgaaa 180

tctgtcgatg aggttagagg aacggatact ccaccggaga aggttccgcg tcgtgtcctg 240

ccgtctggat ttaagccggc tgaatccgcc ggtgatgctt cgtccctggt ctccaatatt 300

atgcataagt ttgtaaaagt cgatgatcga gattgttctg gagagaggag ccgagaagat 360

gttgttccgc tgaatgattc atctctatgt atgaaggcta atgatgttat tcctcaattt 420

cgttccaata atggtaaaac tcaagaaaga aaccatgctt ttagtttcag tgggagagct 480

gaacttagat cagtagaaga tataggagta gatggcgatg ttcttggtcc agaaacacca 540

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gaggataagg ttctgtatt ggactctaac aaaaggctga aaatgctcca ggatccgggtt 660

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<210> 28
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<210> 29
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<220>
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Met Gln Arg Gln Arg Ser Ile Leu Ser Phe
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23

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Phe Gln Lys Pro Thr Ala Ala Thr Thr Lys Gly Leu Val Ser Gly Asp	
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Ala Ala Ser Gly Gly Gly Gly Ser Gly Gly Pro Arg Phe Asn Val Arg	
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gaa ggg gat gct aaa ggc gac gct tct gta cgt ttt gct gtt tcg aaa	315
Glu Gly Asp Ala Lys Gly Asp Ala Ser Val Arg Phe Ala Val Ser Lys	
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tct gtc gat gag gtt aga gga acg gat act cca ccg gag aag gtt ccg	363
Ser Val Asp Glu Val Arg Gly Thr Asp Thr Pro Pro Glu Lys Val Pro	
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Arg Arg Val Leu Pro Ser Gly Phe Lys Pro Ala Glu Ser Ala Gly Asp	
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Asn Asp Ser Ser Leu Cys Met Lys Ala Asn Asp Val Ile Pro Gln Phe	
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Leu Lys Arg Val Leu Glu Asp Glu Met Thr Phe Lys Glu Asp Lys Val	
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Pro Val Leu Asp Ser Asn Lys Arg Leu Lys Met Leu Gln Asp Pro Val	
205 210 215	

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Cys Gly Glu Lys Lys Glu Val Asn Glu Gly Thr Lys Phe Glu Trp Leu	
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Leu Tyr Asp Arg Lys Thr Leu His Ile Pro Pro Asp Val Phe Lys Lys	
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Met Ser Ala Ser Gln Lys Lys Tyr Trp Ser Val Lys Ser Glu Tyr Met	
270 275 280	
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25

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Ala Leu Arg Lys Tyr Thr Leu Thr Gly Ser Thr Ala Val Gln Leu Ala	
460 465 470	
cca gta cca caa gta atg ggg gat aca gat gct gct gga gtt aga aat	1611
Pro Val Pro Gln Val Met Gly Asp Thr Asp Ala Ala Gly Val Arg Asn	
475 480 485 490	
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Ser Pro Thr Gly Lys Arg Leu Leu Arg Asn Trp Ile Cys His Pro Leu	
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Lys Asp Val Glu Ser Ile Asn Lys Arg Leu Asp Val Val Glu Glu Phe	
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Thr Ala Asn Ser Glu Ser Met Gln Ile Thr Gly Gln Tyr Leu His Lys	
620 625 630	

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Gln Arg Val Lys Ala Phe Gly Gln Ile Val Lys Gly Phe Arg Ser Gly	
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715 720 725 730	
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765 770 775	
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795 800 805 810	
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cct gtt ccg aat gat ata ctc ctt ggc gag gct aga aga agc agt ggc	2667
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830 835 840	

agc att cat cct cgg tca ttg tta ctg acg gga cca aac atg ggc gga	2715
Ser Ile His Pro Arg Ser Leu Leu Leu Thr Gly Pro Asn Met Gly Gly	
845 850 855	
aaa tca act ctt ctt cgt gca aca tgt ctg gcc gtt atc ttt gcc caa	2763
Lys Ser Thr Leu Leu Arg Ala Thr Cys Leu Ala Val Ile Phe Ala Gln	
860 865 870	
ctt ggc tgc tac gtg ccg tgt gag tct tgc gaa atc tcc ctc gtg gat	2811
Leu Gly Cys Tyr Val Pro Cys Glu Ser Cys Glu Ile Ser Leu Val Asp	
875 880 885 890	
act atc ttc aca agg ctt ggc gca tct gat aga atc atg aca gga gag	2859
Thr Ile Phe Thr Arg Leu Gly Ala Ser Asp Arg Ile Met Thr Gly Glu	
895 900 905	
agt acc ttt ttg gta gaa tgc act gag aca gcg tca gtt ctt cag aat	2907
Ser Thr Phe Leu Val Glu Cys Thr Glu Thr Ala Ser Val Leu Gln Asn	
910 915 920	
gca act cag gat tca cta gta atc ctt gac gaa ctg ggc aga gga act	2955
Ala Thr Gln Asp Ser Leu Val Ile Leu Asp Glu Leu Gly Arg Gly Thr	
925 930 935	
agt act ttc gat gga tac gcc att gca tac tcg gtt ttt cgt cac ctg	3003
Ser Thr Phe Asp Gly Tyr Ala Ile Ala Tyr Ser Val Phe Arg His Leu	
940 945 950	
gta gag aaa gtt caa tgt cgg atg ctc ttt gca aca cat tac cac cct	3051
Val Glu Lys Val Gln Cys Arg Met Leu Phe Ala Thr His Tyr His Pro	
955 960 965 970	
ctc acc aag gaa ttc gcg tct cac cca cgt gtc acc tcg aaa cac atg	3099
Leu Thr Lys Glu Phe Ala Ser His Pro Arg Val Thr Ser Lys His Met	
975 980 985	
gct tgc gca ttc aaa tca aga tct gat tat caa cca cgt ggt tgt gat	3147
Ala Cys Ala Phe Lys Ser Arg Ser Asp Tyr Gln Pro Arg Gly Cys Asp	
990 995 1000	
caa gac cta gtg ttc ttg tac cgt tta acc gag gga gct tgt cct gag	3195
Gln Asp Leu Val Phe Leu Tyr Arg Leu Thr Glu Gly Ala Cys Pro Glu	
1005 1010 1015	
agc tac gga ctt caa gtg gca ctc atg gct gga ata cca aac caa gtg	3243
Ser Tyr Gly Leu Gln Val Ala Leu Met Ala Gly Ile Pro Asn Gln Val	
1020 1025 1030	
ggt gaa aca gca tca ggt gct gct caa gcc atg aag aga tca att ggg	3291
Val Glu Thr Ala Ser Gly Ala Ala Gln Ala Met Lys Arg Ser Ile Gly	
1035 1040 1045 1050	

28

gga aac ttc aag tca agt gag cta aga tct gag ttc tca agt ctg cat 3339
 Glu Asn Phe Lys Ser Ser Glu Leu Arg Ser Glu Phe Ser Ser Leu His
 1055 1060 1065

gaa gac tgg ctc aag tca ttg gtg ggt att tct cga gtc gcc cac aac 3387
 Glu Asp Trp Leu Lys Ser Leu Val Gly Ile Ser Arg Val Ala His Asn
 1070 1075 1080

aat gcc ccc att ggc gaa gat gac tac gac act ttg ttt tgc tta tgg 3435
 Asn Ala Pro Ile Gly Glu Asp Asp Tyr Asp Thr Leu Phe Cys Leu Trp
 1085 1090 1095

cat gag atc aaa tcc tct tac tgt gtt ccc aaa taaatggcta 3478
 His Glu Ile Lys Ser Ser Tyr Cys Val Pro Lys
 1100 1105

tgacataaca ctatctgaag ctcgttaagt cttttgcctc tctgatgttt attcctctta 3538

aaaaatgctt atatatcaaa aaattgtttc ctcgattaaa aaaaaaaaaa aaaaaaaaaa 3598

aaaaaaaaaa 3606

<210> 31
 <211> 1109
 <212> PRT
 <213> *Arabidopsis thaliana* ecotype Columbia
 <223> Polypeptide MSH6

<400> 31

Met Gln Arg Gln Arg Ser Ile Leu Ser Phe Phe Gln Lys Pro Thr Ala
 1 5 10 15

Ala Thr Thr Lys Gly Leu Val Ser Gly Asp Ala Ala Ser Gly Gly Gly
 20 25 30

Gly Ser Gly Gly Pro Arg Phe Asn Val Arg Glu Gly Asp Ala Lys Gly
 35 40 45

Asp Ala Ser Val Arg Phe Ala Val Ser Lys Ser Val Asp Glu Val Arg
 50 55 60

Gly Thr Asp Thr Pro Pro Glu Lys Val Pro Arg Arg Val Leu Pro Ser
 65 70 75 80

Gly Phe Lys Pro Ala Glu Ser Ala Gly Asp Ala Ser Ser Leu Phe Ser
 85 90 95

Asn Ile Met His Lys Phe Val Lys Val Asp Asp Arg Asp Cys Ser Gly
 100 105 110

Glu Arg Ser Arg Glu Asp Val Val Pro Leu Asn Asp Ser Ser Leu Cys
 115 120 125

Met	Lys	Ala	Asn	Asp	Val	Ile	Pro	Gln	Phe	Arg	Ser	Asn	Asn	Gly	Lys	130	135	140
Thr	Gln	Glu	Arg	Asn	His	Ala	Phe	Ser	Phe	Ser	Gly	Arg	Ala	Glu	Leu	145	150	155
Arg	Ser	Val	Glu	Asp	Ile	Gly	Val	Asp	Gly	Asp	Val	Pro	Gly	Pro	Glu	165	170	175
Thr	Pro	Gly	Met	Arg	Pro	Arg	Ala	Ser	Arg	Leu	Lys	Arg	Val	Leu	Glu	180	185	190
Asp	Glu	Met	Thr	Phe	Lys	Glu	Asp	Lys	Val	Pro	Val	Leu	Asp	Ser	Asn	195	200	205
Lys	Arg	Leu	Lys	Met	Leu	Gln	Asp	Pro	Val	Cys	Gly	Glu	Lys	Lys	Glu	210	215	220
Val	Asn	Glu	Gly	Thr	Lys	Phe	Glu	Trp	Leu	Glu	Ser	Ser	Arg	Ile	Arg	225	230	235
Asp	Ala	Asn	Arg	Arg	Arg	Pro	Asp	Asp	Pro	Leu	Tyr	Asp	Arg	Lys	Thr	245	250	255
Leu	His	Ile	Pro	Pro	Asp	Val	Phe	Lys	Lys	Met	Ser	Ala	Ser	Gln	Lys	260	265	270
Gln	Tyr	Trp	Ser	Val	Lys	Ser	Glu	Tyr	Met	Asp	Ile	Val	Leu	Phe	Phe	275	280	285
Lys	Val	Gly	Lys	Phe	Tyr	Glu	Leu	Tyr	Glu	Leu	Asp	Ala	Glu	Leu	Gly	290	295	300
His	Lys	Glu	Leu	Asp	Trp	Lys	Met	Thr	Met	Ser	Gly	Val	Gly	Lys	Cys	305	310	315
Arg	Gln	Val	Gly	Ile	Ser	Glu	Ser	Gly	Ile	Asp	Glu	Ala	Val	Gln	Lys	325	330	335
Leu	Leu	Ala	Arg	Gly	Tyr	Lys	Val	Gly	Arg	Ile	Glu	Gln	Leu	Glu	Thr	340	345	350
Ser	Asp	Gln	Ala	Lys	Ala	Arg	Gly	Ala	Asn	Thr	Ile	Ile	Pro	Arg	Lys	355	360	365
Leu	Val	Gln	Val	Leu	Thr	Pro	Ser	Thr	Ala	Ser	Glu	Gly	Asn	Ile	Gly	370	375	380
Pro	Asp	Ala	Val	His	Leu	Leu	Ala	Ile	Lys	Glu	Ile	Lys	Met	Glu	Leu	385	390	395
Gln	Lys	Cys	Ser	Thr	Val	Tyr	Gly	Phe	Ala	Phe	Val	Asp	Cys	Ala	Ala	405	410	415

Leu Arg Phe Trp Val Gly Ser Ile Ser Asp Asp Ala Ser Cys Ala Ala
 420 425 430
 Leu Gly Ala Leu Leu Met Gln Val Ser Pro Lys Glu Val Leu Tyr Asp
 435 440 445
 Ser Lys Gly Leu Ser Arg Glu Ala Gln Lys Ala Leu Arg Lys Tyr Thr
 450 455 460
 Leu Thr Gly Ser Thr Ala Val Gln Leu Ala Pro Val Pro Gln Val Met
 465 470 475 480
 Gly Asp Thr Asp Ala Ala Gly Val Arg Asn Ile Ile Glu Ser Asn Gly
 485 490 495
 Tyr Phe Lys Gly Ser Ser Glu Ser Trp Asn Cys Ala Val Asp Gly Leu
 500 505 510
 Asn Glu Cys Asp Val Ala Leu Ser Ala Leu Gly Glu Leu Ile Asn His
 515 520 525
 Leu Ser Arg Leu Lys Leu Glu Asp Val Leu Lys His Gly Asp Ile Phe
 530 535 540
 Pro Tyr Gln Val Tyr Arg Gly Cys Leu Arg Ile Asp Gly Gln Thr Met
 545 550 555 560
 Val Asn Leu Glu Ile Phe Asn Asn Ser Cys Asp Gly Gly Pro Ser Gly
 565 570 575
 Thr Leu Tyr Lys Tyr Leu Asp Asn Cys Val Ser Pro Thr Gly Lys Arg
 580 585 590
 Leu Leu Arg Asn Trp Ile Cys His Pro Leu Lys Asp Val Glu Ser Ile
 595 600 605
 Asn Lys Arg Leu Asp Val Val Glu Glu Phe Thr Ala Asn Ser Glu Ser
 610 615 620
 Met Gln Ile Thr Gly Gln Tyr Leu His Lys Leu Pro Asp Leu Glu Arg
 625 630 635 640
 Leu Leu Gly Arg Ile Lys Ser Ser Val Arg Ser Ser Ala Ser Val Leu
 645 650 655
 Pro Ala Leu Leu Gly Lys Lys Val Leu Lys Gln Arg Val Lys Ala Phe
 660 665 670
 Gly Gln Ile Val Lys Gly Phe Arg Ser Gly Ile Asp Leu Leu Leu Ala
 675 680 685
 Leu Gln Lys Glu Ser Asn Met Met Ser Leu Leu Tyr Lys Leu Cys Lys
 690 695 700

Leu Pro Ile Leu Val Gly Lys Ser Gly Leu Glu Leu Phe Leu Ser Gln
 705 710 715 720
 Phe Glu Ala Ala Ile Asp Ser Asp Phe Pro Asn Tyr Gln Asn Gln Asp
 725 730 735
 Val Thr Asp Glu Asn Ala Glu Thr Leu Thr Ile Leu Ile Glu Leu Phe
 740 745 750
 Ile Glu Arg Ala Thr Gln Trp Ser Glu Val Ile His Thr Ile Ser Cys
 755 760 765
 Leu Asp Val Leu Arg Ser Phe Ala Ile Ala Ala Ser Leu Ser Ala Gly
 770 775 780
 Ser Met Ala Arg Pro Val Ile Phe Pro Glu Ser Glu Ala Thr Asp Gln
 785 790 795 800
 Asn Gln Lys Thr Lys Gly Pro Ile Leu Lys Ile Gln Gly Leu Trp His
 805 810 815
 Pro Phe Ala Val Ala Ala Asp Gly Gln Leu Pro Val Pro Asn Asp Ile
 820 825 830
 Leu Leu Gly Glu Ala Arg Arg Ser Ser Gly Ser Ile His Pro Arg Ser
 835 840 845
 Leu Leu Leu Thr Gly Pro Asn Met Gly Gly Lys Ser Thr Leu Leu Arg
 850 855 860
 Ala Thr Cys Leu Ala Val Ile Phe Ala Gln Leu Gly Cys Tyr Val Pro
 865 870 875 880
 Cys Glu Ser Cys Glu Ile Ser Leu Val Asp Thr Ile Phe Thr Arg Leu
 885 890 895
 Gly Ala Ser Asp Arg Ile Met Thr Gly Glu Ser Thr Phe Leu Val Glu
 900 905 910
 Cys Thr Glu Thr Ala Ser Val Leu Gln Asn Ala Thr Gln Asp Ser Leu
 915 920 925
 Val Ile Leu Asp Glu Leu Gly Arg Gly Thr Ser Thr Phe Asp Gly Tyr
 930 935 940
 Ala Ile Ala Tyr Ser Val Phe Arg His Leu Val Glu Lys Val Gln Cys
 945 950 955 960
 Arg Met Leu Phe Ala Thr His Tyr His Pro Leu Thr Lys Glu Phe Ala
 965 970 975
 Ser His Pro Arg Val Thr Ser Lys His Met Ala Cys Ala Phe Lys Ser
 980 985 990

32

Arg Ser Asp Tyr Gln Pro Arg Gly Cys Asp Gln Asp Leu Val Phe Leu
 995 1000 1005

Tyr Arg Leu Thr Glu Gly Ala Cys Pro Glu Ser Tyr Gly Leu Gln Val
 1010 1015 1020

Ala Leu Met Ala Gly Ile Pro Asn Gln Val Val Glu Thr Ala Ser Gly
 1025 1030 1035 1040

Ala Ala Gln Ala Met Lys Arg Ser Ile Gly Glu Asn Phe Lys Ser Ser
 1045 1050 1055

Glu Leu Arg Ser Glu Phe Ser Ser Leu His Glu Asp Trp Leu Lys Ser
 1060 1065 1070

Leu Val Gly Ile Ser Arg Val Ala His Asn Asn Ala Pro Ile Gly Glu
 1075 1080 1085

Asp Asp Tyr Asp Thr Leu Phe Cys Leu Trp His Glu Ile Lys Ser Ser
 1090 1095 1100

Tyr Cys Val Pro Lys
 1105

<210> 32
 <211> 24
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Forward primer for PCR amplification of ATHGENEA
 microsatellite

<400> 32

accatgcata gcttaaactt cttg

24

<210> 33
 <211> 22
 <212> DNA
 <213> Artificial sequence

<220>
 <223> Reverse primer for PCR amplification of ATHGENEA
 microsatellite

<400> 33

acataaccac aaataggggt gc

22

<210> 34
 <211> 18
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Forward primer DMCIN-A for PCR on genomic DNA of *Arabidopsis thaliana* ssp. *Landsberg erecta* "Ler"

 <400> 34
 gaagcgatat tggttcgtg 18

 <210> 35
 <211> 18
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Reverse primer DMCIN-B for PCR on genomic DNA of *Arabidopsis thaliana* ssp. *Landsberg erecta* "Ler"

 <400> 35
 agattgcgag aacattcc 18

 <210> 36
 <211> 31
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Forward primer DMCIN-1 for PCR on genomic DNA of *Arabidopsis thaliana* ssp. *Landsberg erecta* "Ler"

 <400> 36
 acgcgtcgac tcagctatga gattactcgt g 31

 <210> 37
 <211> 29
 <212> DNA
 <213> Artificial sequence

 <220>
 <223> Reverse primer DMCIN-2 for PCR on genomic DNA of *Arabidopsis thaliana* ssp. *Landsberg erecta* "Ler"

 <400> 37
 gctctagatt tctcgtctcta agactctct 29

<210> 38
<211> 32
<212> DNA
<213> Artificial sequence

<220>
<223> Forward primer DMCIN-3 for PCR on genomic DNA of Arabidopsis thaliana ssp. Landsberg erecta "Ler"

<400> 38

gctctagagc ttctcttaag taagtgattg at 32

<210> 39
<211> 48
<212> DNA
<213> Artificial sequence

<220>
<223> Reverse primer DMCIN-4 for PCR on genomic DNA of Arabidopsis thaliana ssp. Landsberg erecta "Ler"

<400> 39

tcccccgggc tcgagagatc tccatgggtt cttcagctct atgaatcc 48

<210> 40
<211> 26
<212> DNA
<213> Artificial sequence

<220>
<223> Forward primer DMC1a for PCR on genomic DNA of Arabidopsis thaliana ssp. Landsberg erecta "Ler"

<400> 40

acgcgtcgac gaattcgcaa gtgggg 26

<210> 41
<211> 38
<212> DNA
<213> Artificial sequence

<220>
<223> Reverse primer DMC1b for PCR on genomic DNA of Arabidopsis thaliana ssp. Landsberg erecta "Ler"

<400> 41

tccatggaga tctcccggtt accgatttgc ttcgaggg

38

<210> 42
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Forward primer for PCR amplification of ATEAT1 SSLP marker in
Arabidopsis thaliana subspecies

<400> 42

gccactgcgt gaatgatatg

20

<210> 43
<211> 22
<212> DNA
<213> Artificial sequence

<220>
<223> Reverse primer for PCR amplification of ATEAT1 SSLP marker in
Arabidopsis thaliana subspecies

<400> 43

cgaacagcca acattaattc cc

22

<210> 44
<211> 18
<212> DNA
<213> Artificial sequence

<220>
<223> Forward primer for PCR amplification of NGA63 SSLP marker in
Arabidopsis thaliana subspecies

<400> 44

aaccaaggca cagaagcg

18

<210> 45
<211> 18
<212> DNA
<213> Artificial sequence

<220>
<223> Reverse primer for PCR amplification of NGA63 SSLP marker in
Arabidopsis thaliana subspecies

<400> 45

acccaagtga tcgccacc

18

<210> 46

<211> 21

<212> DNA

<213> Artificial sequence

<220>

<223> Forward primer for PCR amplification of NGA248 SSLP marker in Arabidopsis thaliana subspecies

<400> 46

taccgaacca aaacacaaag g

21

<210> 47

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Reverse primer for PCR amplification of NGA248 SSLP marker in Arabidopsis thaliana subspecies

<400> 47

tctgtatctc ggtgaattct cc

22

<210> 48

<211> 22

<212> DNA

<213> Artificial sequence

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<223> Forward primer for PCR amplification of NGA128 SSLP marker in Arabidopsis thaliana subspecies

<400> 48

ggtctgttga tgtcgtaagt cg

22

<210> 49

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Reverse primer for PCR amplification of NGA128 SSLP marker in
Arabidopsis thaliana subspecies

<400> 49

atcttgaaac ctttagggag gg 22

<210> 50
<211> 22
<212> DNA
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<220>
<223> Forward primer for PCR amplification of NGA280 SSLP marker in
Arabidopsis thaliana subspecies

<400> 50

ctgatctcac ggacaatagt gc 22

<210> 51
<211> 20
<212> DNA
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<220>
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Arabidopsis thaliana subspecies

<400> 51

ggctccataa aaagtgcacc 20

<210> 52
<211> 21
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<220>
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Arabidopsis thaliana subspecies

<400> 52

ctccagttgg aagctaaagg g 21

<210> 53
<211> 21
<212> DNA
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<220>
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Arabidopsis thaliana subspecies

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tggttttttag gacaaatggc g 21

<210> 54
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<212> DNA
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<220>
<223> Forward primer for PCR amplification of NGA168 SSLP marker in
Arabidopsis thaliana subspecies

<400> 54
ccttcacatc caaaacccac 20

<210> 55
<211> 20
<212> DNA
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<220>
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Arabidopsis thaliana subspecies

<400> 55
gcacataccc acaaccagaa 20

<210> 56
<211> 20
<212> DNA
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<220>
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in Arabidopsis thaliana subspecies

<400> 56
cgctacgctt ttcggtaaag 20

<210> 57
<211> 20
<212> DNA
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<220>
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in Arabidopsis thaliana subspecies

<400> 57

gcacagtcca agtcacaacc

20

<210> 58
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Forward primer for PCR amplification of NGA361 SSLP marker in
Arabidopsis thaliana subspecies

<400> 58

aaagagatga gaatttggac

20

<210> 59
<211> 23
<212> DNA
<213> Artificial sequence

<220>
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Arabidopsis thaliana subspecies

<400> 59

acatatcaat atattaaagt agc

23

<210> 60
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<212> DNA
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<220>
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Arabidopsis thaliana subspecies

<400> 60

tcgtctactg cactgccg

18

<210> 61
<211> 22
<212> DNA
<213> Artificial sequence

<220>
<223> Reverse primer for PCR amplification of NGA168 SSLP marker in
Arabidopsis thaliana subspecies

<400> 61

gaggacatgt ataggagcct cg

22

<210> 62
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Forward primer for PCR amplification of AthBIO2 SSLP marker
in Arabidopsis thaliana subspecies

<400> 62

tgacctcctc ttccatggag

20

<210> 63
<211> 22
<212> DNA
<213> Artificial sequence

<220>
<223> Reverse primer for PCR amplification of AthBIO2 SSLP marker
in Arabidopsis thaliana subspecies

<400> 63

ttaacagaaa cccaaagctt tc

22

<210> 64
<211> 21
<212> DNA
<213> Artificial sequence

<220>
<223> Forward primer for PCR amplification of AthUBIQUE SSLP marker
in Arabidopsis thaliana subspecies

<400> 64
aggcaaagt ccatttcatt g 21

<210> 65
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Reverse primer for PCR amplification of AthUBIQUE SSLP marker
in Arabidopsis thaliana subspecies

<400> 65
acgacatggc agatttctcc 20

<210> 66
<211> 21
<212> DNA
<213> Artificial sequence

<220>
<223> Forward primer for PCR amplification of NGA172 SSLP marker in
Arabidopsis thaliana subspecies

<400> 66
agctgcttcc ttatagcgtc c 21

<210> 67
<211> 19
<212> DNA
<213> Artificial sequence

<220>
<223> Reverse primer for PCR amplification of NGA172 SSLP marker in
Arabidopsis thaliana subspecies

<400> 67
catccgaatg ccattgttc 19

<210> 68
<211> 21
<212> DNA
<213> Artificial sequence

<220>

<223> Forward primer for PCR amplification of NGA126 SSLP marker in
Arabidopsis thaliana subspecies

<400> 63

gaaaaaacgc tacttttcgtg g 21

<210> 69
<211> 22
<212> DNA
<213> Artificial sequence

<220>
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Arabidopsis thaliana subspecies

<400> 69

caagagcaat atcaagagca gc 22

<210> 70 -
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<212> DNA
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<220>
<223> Forward primer for PCR amplification of NGA162 SSLP marker in
Arabidopsis thaliana subspecies

<400> 70

catgcaattt gcatctgagg 20

<210> 71
<211> 22
<212> DNA
<213> Artificial sequence

<220>
<223> Reverse primer for PCR amplification of NGA162 SSLP marker in
Arabidopsis thaliana subspecies

<400> 71

ctctgtcact cttttctctt gg 22

<210> 72
<211> 21
<212> DNA
<213> Artificial sequence

<220>
<223> Forward primer for PCR amplification of NGA6 SSLP marker in
Arabidopsis thaliana subspecies

<400> 72

tggaatttttt cctctctttca c 21

<210> 73
<211> 21
<212> DNA
<213> Artificial sequence

<220>
<223> Reverse primer for PCR amplification of NGA6 SSLP marker in
Arabidopsis thaliana subspecies

<400> 73

atggagaagc ttacactgat c 21

<210> 74
<211> 20
<212> DNA
<213> Artificial sequence

<220>
<223> Forward primer for PCR amplification of NGA12 SSLP marker in
Arabidopsis thaliana subspecies

<400> 74

aatggttgccc tccccctctc 20

<210> 75
<211> 22
<212> DNA
<213> Artificial sequence

<220>
<223> Reverse primer for PCR amplification of NGA12 SSLP marker in
Arabidopsis thaliana subspecies

<400> 75

tgatgctctc tgaacaaga gc 22

<210> 76
<211> 21
<212> DNA
<213> Artificial sequence

<220>
<223> Forward primer for PCR amplification of NGA8 SSLP marker in
Arabidopsis thaliana subspecies

<400> 76

gagggcaaat ctttatttcg g

21

<210> 77
<211> 22
<212> DNA
<213> Artificial sequence

<220>
<223> Reverse primer for PCR amplification of NGA8 SSLP marker in
Arabidopsis thaliana subspecies

<400> 77

tggctttcgt ttataaacat cc

22

<210> 78
<211> 21
<212> DNA
<213> Artificial sequence

<220>
<223> Forward primer for PCR amplification of NGA1107 SSLP marker
in Arabidopsis thaliana subspecies

<400> 78

gcgaaaaaac aaaaaaatcc a

21

<210> 79
<211> 21
<212> DNA
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<220>
<223> Reverse primer for PCR amplification of NGA1107 SSLP marker
in Arabidopsis thaliana subspecies

<400> 79

cgacgaatcg acagaattag.g

21

<210> 80
<211> 21
<212> DNA
<213> Artificial sequence

<220>
<223> Forward primer for PCR amplification of NGA225 SSLP marker in
Arabidopsis thaliana subspecies

<400> 80

gaaatccaaa tcccagagag g

21

<210> 81
<211> 22
<212> DNA
<213> Artificial sequence

<220>
<223> Reverse primer for PCR amplification of NGA225 SSLP marker in
Arabidopsis thaliana subspecies

<400> 81

tctccccact agttttgtgt cc

22

<210> 82
<211> 19
<212> DNA
<213> Artificial sequence

<220>
<223> Forward primer for PCR amplification of NGA249 SSLP marker in
Arabidopsis thaliana subspecies

<400> 82

taccgtcaat ttcacgccc

19

<210> 83
<211> 22
<212> DNA
<213> Artificial sequence

<220>
<223> Reverse primer for PCR amplification of NGA249 SSLP marker in
Arabidopsis thaliana subspecies

<400> . . 83 .

ggatccctaa ctgtaaaatc cc

22

<210> 84

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Forward primer for PCR amplification of CA72 SSLP marker in
Arabidopsis thaliana subspecies

<400> 84

aatcccagta accaaacaca ca

22

<210> 85

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Reverse primer for PCR amplification of CA72 SSLP marker in
Arabidopsis thaliana subspecies

<400> 85

cccagtctaa ccacgaccac

20

<210> 86

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Forward primer for PCR amplification of NGA151 SSLP marker in
Arabidopsis thaliana subspecies

<400> 86

gttttgaggaa gttttgctgg

20

<210> 87

<211> 24

<212> DNA

<213> Artificial sequence

<220>

<223> Reverse primer for PCR amplification of NGA151 SSLP marker in
Arabidopsis thaliana subspecies

<400> 87

cagtctaaaa gcgagagtat gatg

24

<210> 88

<211> 22

<212> DNA

<213> Artificial sequence

<220>

<223> Forward primer for PCR amplification of NGA106 SSLP marker in
Arabidopsis thaliana subspecies

<400> 88

gttatggagt ttctagggca cg

22

<210> 89

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Reverse primer for PCR amplification of NGA106 SSLP marker in
Arabidopsis thaliana subspecies

<400> 89

tgccccatctt tgttcttctc

20

<210> 90

<211> 20

<212> DNA

<213> Artificial sequence

<220>

<223> Forward primer for PCR amplification of NGA139 SSLP marker in
Arabidopsis thaliana subspecies

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